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THE EFFICACY OF CERTAIN SUBSTITUTED PHENOLS AND THEIR SALTS AS FUNGICIDAL AGENTS[1]

By GENE M. LE FAVE[2] AND BRUCE L. SKILES

Amine addition products of polychlorophenols have formed the subject of a large number of patents but have not been exploited to any extent as fungicides of commerce.

We have prepared a series of various amine salts of polychlorophenols utilizing, for the most part, pentachlorophenol. The majority of the compounds prepared are new although a few have appeared in the patent literature (1). A few copper compounds were also prepared along with some interesting miscellaneous phenolics. The more promising materials were selected and evaluated for their mildew-proofing properties in textiles by the fungus mat method (2) and in paint by the recently developed accelerated method of Vicklund, *et al* (3).

PREPARATION AND PROPERTIES OF THE AMINE PENTACHLOROPHENATES

Pure pentachlorophenol was obtained by an efficient steam distillation of the technical product and recrystallization from carbon tetrachloride. Other phenols used were obtained commercially in a stated purity of 98% or higher. The amines were obtained with a minimum purity of 98% or were purified to that extent. The fatty amines were supplied gratuitously by Dr. L. Armstrong of the Armour Company and used without further purification.

The majority of the compounds were prepared simply by warming a molecular equivalent each of the amine and phenol in a mutual solvent such as methanol and allowing the product to crystallize or

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[1] Presented before the Pesticide Section of the XIIth International Congress of Pure and Applied Chemistry, Paper No. 18, September 12, 1951. A considerable amount of the evaluation work represents a portion of a thesis submitted by Mr. Skiles in partial fulfillment of the requirements for the Master of Science degree in the Division of Graduate Instruction, Butler University.

[2] J. I. Holcomb Research Laboratories, Indianapolis 8, Indiana.

stripping the solvent if a liquid. An alternative method was occasionally employed; aqueous solutions of the amine hydrochloride and sodium phenate were mixed, precipitating the amine phenate metathetically.

Of the amine polychlorophenates prepared the majority are white crystalline solids although some are heavy oils. They are freely soluble in most organic solvents and relatively insoluble in water. Some, such as the pyridine, acridine and nicotine addition products appear to be molecular complexes resembling hydrocarbon picrates rather than true salts. These complexes, like the picrates, are quite stable.

FUNGICIDAL EVALUATION

All compounds were screened initially against *Alternaria solani* by the modified spore-germination method of Peterson (4), the results of which were found to be reproducible within an error of five per cent at a spore concentration of 5×10^4 / ml.

Results of the screening tests appear in Tables I, II, III, and IV. We believe, that considering the limitations imposed by the genestatic approach to laboratory assay of fungicides, the results are reliable. Subsequent mildew-proofing tests employing the fungicides found superior by the Peterson method support this view in part. The reliability would obviously be vitiated upon variation of fungus species and chemical type of fungicide.

It is apparent from the screening data that the fatty amine addition products of the chlorinated phenols are quite superior as fungicides toward sporulating fungi. The highest range of activity lies between ten and sixteen carbon atoms. The lower molecular weight amine salts in general appear to be equal or inferior to pentachlorophenol alone. Branching of the straight chain aliphatic amines reduces the potency of the salt.

An explanation of the high order of activity of the fatty amine salts of the substituted phenols may be found in the fatty amines themselves. They were found to possess an exceptionally high antifungal activity which will comprise the subject of a future report to be published elsewhere.

It is of passing interest to note that the majority of the amine pentachlorophenates, on the basis of their infra-red patterns in the hydrogen-bond region, show almost complete dissociation.

We find it rather difficult to account for the activity of the aminopropionitrile salts since the aminopropionitriles possess no fungicidal activity whatever. Perhaps a synergistic action is involved. Indeed, synergism is operative in a large number of the amine salts when the proportional molecular weight contribution of the phenolic moity is considered.

EVALUATION OF SELECTED COMPOUNDS AS MILDEW-PROTECTANTS FOR PAINT

By modifying the method of Vicklund and his co-workers (3) slightly it proved entirely satisfactory for our purposes. The aggressive growth characteristics of the organisms used, i.e., *Aspergillus niger, Aspergillus oryzae* 458 and 692, and the simulated tropical environment made it necessary to use a quantity of toxicant amounting to two per cent based on the weight of the paint. Less than this proportion allowed for little differentiation in the amount of growth.

Our observations indicate the following order of effectiveness:

> Copper-8-hydroxyquinolate >> n-Decylamine pentachlorophenate > n-Hexadecylamine pentachlorophenate > "Cocoamine" pentachlorophenate > N-Butyl-*o*-hydroxybenzylimine, copper salt > b, b'-iminodipropionitrile pentachlorophenate > pentachlorophenol > Di-beta-naphthol > butylamine pentachlorophenate.

The characteristic inhibitive properties of most copper compounds appear to operate very efficiently in paint with respect to phenolic compounds. This is in agreement with the findings of Vicklund who used *Aspergilli* as test organisms (3).

EVALUATION OF SELECTED COMPOUNDS FOR MILDEW-PROOFING OF TEXTILES

In Table V are listed the results of mildew-proofing tests carried out against *Chaetomium globosum* according to Marsh and Greathouse (2). The fabric strips were so treated so as to retain 1 ± 0.05% of their weight of toxicant. The breaking strength represents

an average of three strips. The results are in relatively good agreement with what could be expected from the screening data.

SUMMARY

1. A group of amine polychlorophenates were prepared and screened by Peterson's modified spore germination method against *Alternaria solani.*

2. The more promising of these addition products were further screened against other organisms and evaluated as mildew-protectants for paint and textile.

3. The fatty amine pentachlorophenates were found to be very effective mildew-proofing agents on the basis of limited tests.

4. Several miscellaneous copper and phenolic compounds were prepared and screened.

ACKNOWLEDGMENT

We wish to express our appreciation to Dr. Rex Webster for his many helpful suggestions.

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TABLE I

Fungicidal Activity of Amine Pentachlorophenates in Per Cent Germination of *Alternaria Solani* Spores

Amine Salts	Concentration in Weight Per Cent		
	1.0	0.1	0.01
Pentachlorophenol	0	0	90
Butylamine	0	0	93
1,1,3,3-Tetramethylbutylamine	1	17	
Triethylamine	0	8	
Decylamine	0	0	2
Hexadecylamine	0	0	8

TABLE I—(Continued)

Fungicidal Activity of Amine Pentachlorophenates in Per Cent Germination of *Alternaria Solani* Spores

Amine Salts	Concentration in Weight Per Cent 1.0	0.1	0.01
Octadecylamine	2	10	74
3,5,5-Trimethylhexylamine	3	18	
Isopropylamine	0	2	89
Ethylamine	0	0	90
Octylamine	15	65	
Dodecylamine	0	0	2
Tetradecylamine	0	0	13
Morpholine	0	0	89
N-Methylmorpholine	0	0	90
Ethanolamine	0	0	92
Diethanolamine	3	54	
Triethanolamine	0	14	
Tetraethanolammonium	7	53	
Hexamethylenetetramine (Mono)	0	16	80
Pyridine	0	70	
Acridine	13	32	
Nicotine	2	6	71
Triamylamine	0	22	
Phenylhydrazine	6	19	
Thialdine	2	25	
β, β'—Iminodipropionitrile	0	2	8
β-Dimethylaminopropionitrile	0	0	5
β-Isopropylaminopropionitrile	0	0	17
Di-(2-Ethylhexyl)amine	30	74	
N-Methyltaurine	6	20	

TABLE II

Fungicidal Activity of Mixed Amine Polychlorophenates in Per Cent Germination of *Alternaria Solani* Spores

Amine Salts	Concentration in Weight Per Cent 1.0	0.1	0.01
"Cocoamine"[a] Pentachlorophenate (From Coconut Oil)	0	4	22
"Cocoamine" 2,6-Dichloro-4-Nitrophenate	22	40	
"Cocoamine 2,4,6-Trichlorophenate	1	15	86
"Cocoamine" 2,4,5-Trichlorophenate	11	27	
"Cocoamine" Tetrachlorophenate	1	8	45
"Rosin Amine D"[b] Pentachlorophenate	0	7	59
"Sec.-Cocoamine"[a] Pentachlorophenate	26	69	

TABLE II—(Continued)

Fungicidal Activity of Mixed Amine Polychlorophenates in Per Cent Germination of *Alternaria Solani* Spores

Amine Salts	Concentration in Weight Per Cent 1.0	0.1	0.01
"Amine 2HT"[a] Pentachlorophenate (Sec. C_{16} & C_{18} Amines)	17	96	
"Amine TO"[a] Pentachlorophenate (Rosin & Fatty Amine Mixture)	0	1	89
"Alkylamine 81"[c] Pentachlorophenate Tert., Branched, Primary C_{12} to C_{15})	0	45	
"Alkylamine JM"[c] Pentachlorophenate (Tert., Branched, Primary C_{18} Av.)	0	0	31

(a) Armour & Company.
(b) Hercules Powder Co.
(c) Rohm & Haas Company.

TABLE III

Fungicidal Activity of Some Miscellaneous Compounds in Per Cent Germination of *Alternaria Solani* Spores

Compound	Concentration in Weight Per Cent 1.0	0.1	0.01	0.001
Copper-8-Quinolinolate	3	8	15	24
Copper-N-Methyltauride	0	4	97	
Copper-N-Methyltauro-pentachlorophenate	0	9	46	
Copper-2-Mercapto-phenylthiourea	0	10	68	
Undecylenic Acid	0	0	0	96
m-Hydroxybenzotrifluoride	0	0	100	
β-Naphthol	0	0	91	
β-Di-Naphthol	0	0	24	
Hexachlorophene (G-11)	0	3	7	35
N-Butyl-o-Hydroxybenzolimine	93	100		
Copper Salt	0	23	96	
o-Tydroxyphenylbenzothiazole, Copper Salt	0	0	94	
o-Hydroxyphenylbenzothiazole	11	25		
o-Hydrozyphenylbenzoxazole	2	30	100	
Copper Salt	0	63		

TABLE IV

Maximal Concentration Ranges of Certain Amine Pentachlorophenates Required to Inhibit Germination of 50 Per Cent of Spores

Amine Salts	Organism				
	Aspergillus niger	Rhizopus nigricans	Glomerella cingulata	Sclerotinia fructicola	Stemphylium sarcineforme
Pentachlorophenol	0.1-0.01	0.1-0.01	0.01-0.001	0.01-0.001	0.1-0.01
Decylamine Pentachlorophenate	0.01-0.001	0.01-0.001			
Ethanolamine Pentachlorophenate	0.1-0.01	0.01-0.001			
"Cocoamine" Pentachlorophenate	0.01-0.001	0.01-0.001	0.01-0.001	0.01-0.001	0.01-0.001
β, β'-Iminodipropionitrile Pentachlorophenate	0.1-0.01				

TABLE V

The Efficacy of Selected Amine Pentachlorophenates as Textile Mildew-Proofing Against *Chaetonomium globosum*

Compound	Per Cent Residual Strength	Per Cent Residual Strength After 24 Hours Leach at 30° C
Pentachlorophenol	90	30
Decylamine Pentachlorophenate	93	76
Hexadecylamine Pentachlorophenate	91	51
"Rosin Amine D" Pentachlorophenate	86	35
"Cocoamine" Pentachlorophenate	83	54
"Amine TO" Pentachlorophenate	87	30
β, β'-Iminodipropionitrile Pentachlorophenate	95	30

THE BEECH LINE IN NORTHWESTERN INDIANA[1]

By J. E. Potzger and C. O. Keller

In Indiana beech (*Fagus grandifolia*) is without doubt one of the most sensitive indicators of decline in mesophytism in habitat. In the rugged areas of the state it marks the borders between moist north-facing slopes and more xeric south-facing slopes. This was shown by Potzger (1, 2), Potzger and Friesner (3) for the southern as well as for the eastern part of Indiana. Beech also records the effects which the increase of steepness of slope has on the usual more mesic conditions of north-facing slopes. This characteristic of the species suggested a study of the forests along the eastern periphery of our Indiana prairie area to see if the transition between mesophytic forest and prairie functioned as a progressive change or represented a sudden break between the two vegetation types. The senior author is engaged in a study of the original vegetation of the state, using as basis the witness trees noted and recorded by the men who made the original U. S. land survey. The study of distribution of beech along the border of the prairie peninsula is one of several papers dealing with phases of the state-wide survey which seem to warrant more detailed consideration.

METHODS

The information presented here is derived from the records of the original U. S. land survey. All stems of witness trees listed in the counties which mark the western limits of beech were tabulated. The percentage of beech is based on the total number of stems listed, and, in order to accentuate gradual decline in abundance, are given by townships from east to west. Figure 1 is a graphic presentation of these percentages. In order to emphasize the change in forest cover type as one approaches the border of the prairie, a block of

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[1] This is publication 239 of the Botanical Laboratory of Butler University. J. E. Potzger acknowledges with thanks the aid of a Butler University Faculty Fellowship in the preparation of this article. For figure 1 we are indebted to Mrs. Margaret Esther Potzger.

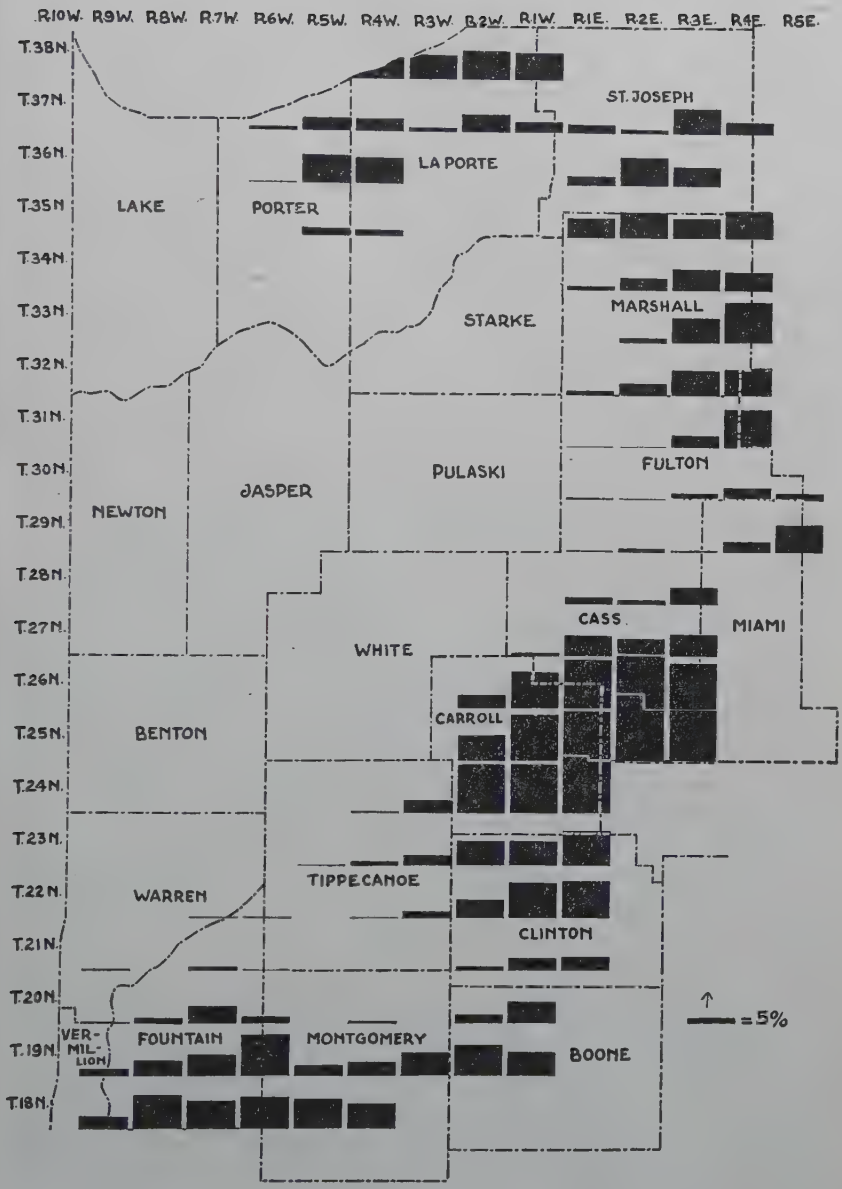

FIGURE 1

Showing progressive decrease of beech as prairie counties are approached. Abundance of beech is shown as percentage of the total number of stems of trees listed by the surveyors for a given township.

24 by 18 miles, comprising most of Marshall County and the eastern section of Starke County, was selected as type location. Percentage representation of the most important mesophytic and xerophytic species in four tiers of 6 by 18 miles is shown in figure 2. The tiers run from R. 1 W., to R. 3 E.

Figure 1 emphasizes the gradual change in forest composition and at the same time the change of habitat from mesophytic forest to grassland. The increasingly less favorable habitat conditions for forest development as one moves westward from upper central Indiana are strikingly expressed by a progressive decrease in abundance of beech, sugar maple (*Acer saccharum*), upland ash (*Fraxinus americana* and *F. quadrangulata*) and other mesophytes in the forest association. The zone is roughly a semicircle extending from Warren County in the south to Porter County in the north (Fig. 1). In the north nearness to Lake Michigan as such does not seem to be so favorable to beech as is the line of prevailing winds crossing the lower tip of Lake Michigan. Decline of beech, maple, and ash in the forest association here is compensated by increase in the number of stems of the more xeric oaks, and, at the transition line, by greater abundance of hickories (Carya). This is shown in figure 2. However, the total number of genera represented in the association complex is also reduced markedly by the dropping out of ash, black walnut (*Juglans nigra*), tulip poplar (*Liriodendron tulipifera*), linden (*Tilia americana*), cherry (Prunus), Carpinus and others. Briefly, the association is now more definitely limited to species of two genera, oak and hickory. However, even here the latter seems to be more selective of an intermediate soil moisture condition which must prevail six miles eastward of the prairie counties.

DISCUSSION

The graphic presentation of figure 1 and the percentage data of figure 2 are so clear that really little need to be added by way of descriptions. The mesophytic forest, which is climatically favored over most of Indiana, is definitely inhibited in Lake, Newton, Benton, Jasper, Starke, Pulaski, and White counties, and is only post- or pre-climax in much of the bordering area. The results of the study definitely indicate that sugar maple, beech, and upland ash species are not only prominent associates in the mesophytic forest, but are indicators of habitat favorable to such a forest association.

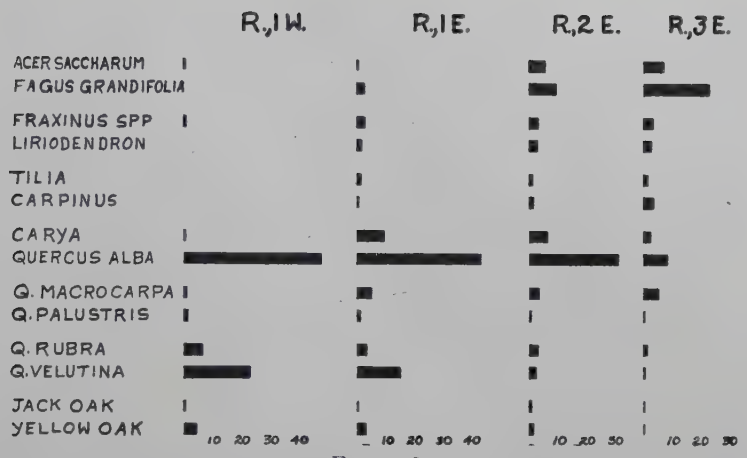

FIGURE 2

Type area (transition) involving a strip 18 miles north and south from eastern Marshall to eastern Starke counties. Showing percentage of the total number of stems listed which a given species represented in the four ranges from east to west.

Factors which eliminate beech and sugar maple also eliminate the major portion of the associates, except the minority oaks and hickories (Fig. 2). In the oak-hickory habitats we find segregation of species. Bur oak (*Quercus macrocarpa*) is an indicator of the wet oak opening forest, white oak (*Q. alba*), black oak (*Q. velutina*), and yellow oak are indicative of the dry oak opening type. (It is impossible to determine from the surveyors' records which species of oak is meant by "yellow oak"). We have here, then, an interesting shuffling of species and genera which cautions against the too liberal use of "oak-hickory" forest cover. Indicated in these "oak-hickory" forests is a major climatic control of oaks and hickories and a microclimatic selection of an edaphic type which segregates certain species of these genera. Only white oak has a range potentiality to encompass all the site conditions favorable to forest occupation. It is also a consistent associate in the mosophytic forest, expanding there in abundance immediately as beech declines (Fig. 2). One can readily understand why by some ecologists the constancy of an association is being doubted or plainly denied, for, as figure 2 shows, we are dealing with merging phenomena. Unless, on the other hand, we assume a rather liberal range in variation of associates in an association we will be forced to flounder in a mass of unrelated individuals. In fact, all group classifications in science would theoretically then have to be

abandoned, for no two individuals of white oak, or of any other species, are absolutely identical.

Upon closer examination one wonders why the prairie influence assumes the geographical position that it does in northwestern Indiana, especially in its limits southwestward, where the break at Vermillion County is very sudden. Also, it penetrates farther eastward than one would theoretically expect, especially at T. 20, and T. 21 and again at T. 28, T. 29, and T. 30 N. It is, of course, beyond the limits of this paper to explain why on the windward side of Lake Michigan nearness to the shore does not terminate its control (Fig. 1).

Even though the continuous presence of beech and sugar maple ends as shown in figure 1, Rohr and Potzger (4) record some isolated disjunct appearances of these species deep within typical prairie in Lake and Newton counties. One would expect this near an ecotone where microclimate may favor both pre-and post-climax establishment of isolated individuals or groups of them. The survey sampling is too large-meshed to give any concept of the number of individuals which may have been involved in the single specimen record, but it does show that these mesophytic species had migrated considerable distance from their limits of continuous distribution.

SUMMARY

1. The study deals with progressive decline of abundance of beech (expressed in terms of percentages) based on total number of stems of all species listed as witness trees in the original U. S. land survey in townships adjacent to the prairie area in northwestern Indiana.

2. Most of the mesophytes in the association complex decline in abundance jointly with beech as one proceeds westward to the prairie border.

3. Species of oak increase in abundance as the prairie border is approached, but the association complex consists of fewer genera than the mesic beech-maple-ash complex.

4. While hickory increases in abundance towards the prairie border, it appears to prefer less xeric habitats than oaks.

5. Beech is a sensitive indicator of decline in soil moisture.

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CHARACTERISTICS OF THE ORIGINAL VEGETATION IN SOME PRAIRIE COUNTIES OF INDIANA[1]

By Dean Finley and J. E. Potzger, Butler University

During the century since civilized man brought great change to the natural vegetation of Indiana by lumbering and farming, the prairie suffered perhaps more than the forest. Agriculture and drainage modified these habitats greatly. An attempt is therefore made to reconstruct the pattern of the original distribution of grasslands in Indiana. Rohr and Potzger (2) described and discussed three northwestern Indiana prairie counties and the present study concerns itself with the prairie-influenced counties to the south and east of those described previously. In the five counties presented in this study the mesophytic forest was almost entirely inhibited and the most favorable areas were occupied by xeric oaks, or oaks and hickories, and even such forests could not always produce a closed canopy.

METHODS

Data were taken from the records of the original U. S. land survey in Warren, Benton, White, Pulaski, and Starke counties. In locations where the surveyors' notes listed posts, the habitat was assumed to have been favorable to prairie, besides, it was usually designated by the surveyors as wet or dry prairie. Figure 1 presents the outline of the counties and the original vegetation patterns. In order to show as a unit the counties in which prairie was conspicuous, we have included in figure 1 the counties described by Rohr and Potzger (2), i. e. Lake, Newton, and Jasper counties.

Distribution of prairie was taken from the surveyors' placement of posts and from their descriptions of the smaller areas rather than

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[1] This is publication 255 of the Botanical Laboratory of Butler University. The senior author expresses his appreciation to Butler University for a Butler Faculty Fellowship which aided in the series of studies concerned with the original vegetation of Indiana. We express our thanks to Mrs. Margaret Esther Potzger for the county outlines and lettering on figure 1.

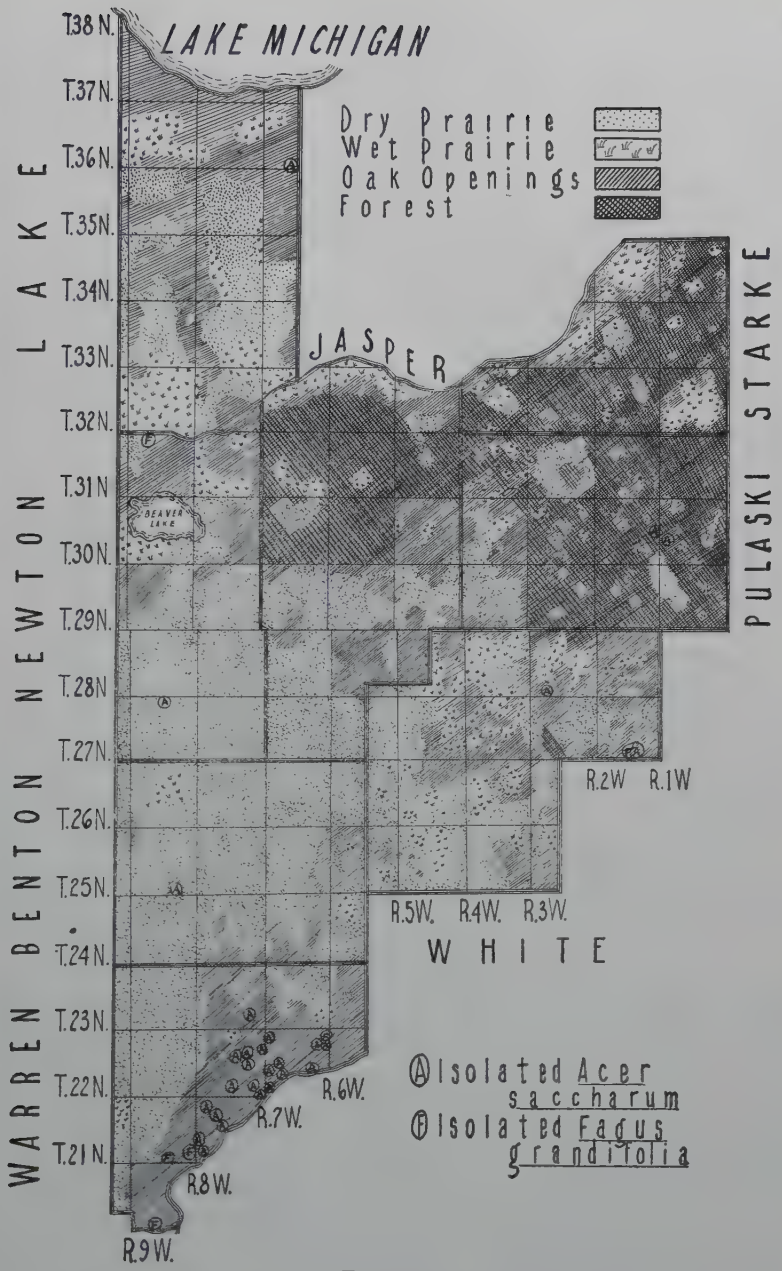

FIGURE 1
Diagrammatic representation of distributional patterns of the original vegetation in eight Indiana "prairie" counties.

from the notes describing whole townships, because the latter appeared to be highly generalized. The oak-opening type forest was based on reduced number of stems listed and on descriptions of the forests.

OBSERVATIONS

The counties included in this study were not all uniform with respect to natural vegetation, as figure 1 plainly shows. Prairie, oak openings and oak forests alternated in larger and smaller areas. At times whole townships were involved in a single "patch." Only Benton County was predominantly prairie. The number of tree species which participated in the forest cover varied greatly too from county to county. Benton 11, White 29, Starke 28, Pulaski 34 and Warren 34. The forest in the whole area was composed chiefly of oaks. Carya (hickory) is significant only in Benton County, while all other counties were 85 to 100 per cent oaks. Abundance of the various species of oaks reflects somewhat their importance in the forest composition:

Species	Benton	Warren	White	Pulaski	Starke
White oak (Quercus alba)	21	263	521	1157	833
Bur oak (Q. macrocarpa)	29	36	41	83	15
Jack oak (Q. ellipsoidalis)	11	14	75	19	—
Black oak (Q. velutina)	11	27	177	770	393
Yellow oak	—	30	30	254	115

Of the northern evergreens Larix was reported for Pulaski and Starke counties. *Thuja occidentalis* for Pulaski County, and Pinus sp. for Starke County (T. 34 N., R. 1 W.).

The most outstanding characteristic of the distribution pattern of prairie and forests is no doubt the result of smaller or larger microclimatic areas, which favor first one and then the other vegetation group. Importance of microclimate is emphasized especially by the invasion of *Acer saccharum* and *Fagus grandifolia*. Outside of a few isolated places, these species invaded along river courses (Fig. 1). Along the western border of the five counties the forest occupies smaller patches of more favorable soil moisture, and along the eastern border the dominance is reversed, here the prairie is distributed in numerous scattered places.

DISCUSSION

For the ecologist there is perhaps no more fascinating pattern of habitats than in an ecotone. Struggle is here emphasized and stately forests may be held in check by grass vegetation, or, at best, they can establish themselves only in small oases of more favorable edaphic conditions. A sharp differentiation is also made between the xeric oaks and hickories and the mesic beech and sugar maple. The latter two species have barely invaded the five counties included in this study. Sugar maple, here too, as in the rugged sections of the state, is more capable than beech to ecese in dry habitats, as Potzger (1) has described for Ripley County.

There is no doubt that forests in the ecotone are usually not so dense as in habitats favoring forests, but it is a little difficult to draw a sharp line between open-crown and closed-crown forests when the bases for such differentiation are mere descriptions or records of witness trees. Nevertheless, Figure 1 makes this differentiation on basis of number of stems recorded in the various townships. In some instances the surveyors made specific mention of the "scattered" tree population, but unfortunately this was not done consistently.

It should, perhaps, be emphasized again that the large areas listed as "wet prairie" very likely were chiefly sedge-meadow. This certainly must have been true along the borders of streams like the Kankakee River. The most significant contribution which this paper aims to make is the pointing out that according to the old survey records the Indiana prairie counties constituted primarily an ecotone between forest and prairie. Extensive areas of prairie were limited to Benton and Newton counties, and about half of Warren and White counties. From here eastward grasslands became more and more fragmented until forest became the controlling vegetation cover. This is more clearly shown in figure 1 than a description could ever accomplish.

SUMMARY

1. The paper presents prairie conditions in five northwestern Indiana counties (Benton, Warren, White, Pulaski, Starke), using as basis the reports of the original U. S. land survey.

2. Wet and dry prairie are mentioned by the surveyors. The probability is expressed that wet prairie may really be sedge-meadow.

3. Outside of Benton, Newton and half of White and Warren counties, distribution of vegetation suggested an ecotone complex rather than that of a prairie. Microclimate very definitely made a heterogeneous selection of forest and prairie patches.

4. Along the southern border of Benton County forests invaded in broad belts along river valleys and to a lesser degree in isolated scattered small patches.

5. Eastward in Pulaski, Starke counties conditions favored the forests and prairie "exploded" into numerous small patches, and forest changed from oak openings to closed-crown forest.

6. Oaks constituted the dominant trees in the five counties, totalling in some areas 85 to 100 per cent of the stems. Oak-hickory association appeared only in the scattered open forests of Benton County.

7. White oak totalled more than 50 per cent of the stems of the several species of oaks represented in the forests.

8. The graphic presentation of distribution of prairie and forest (Fig. 1) clearly portrays the region as one of habitat stresses where microclimate determined distribution of forest and grassland in a highly disjunct pattern.

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CORRELATION OF ELONGATION IN PRIMARY AND SECONDARY BRANCHES OF PINUS RESINOSA

By Ray C. Friesner and J. Johanna Jones

In a previous paper (1) dealing with duration, rate, and magnitude of elongation of primary, secondary, and tertiary axes in two trees of *P. resinosa* and one of *P. strobus,* it was found that: (1) Elongation began at approximately the same time on all branches over the entire contour of the tree. (2) Primary axes showed greater total and higher daily rate of elongation than was shown in any of the secondary or tertiary axes in each of the 3 trees studied. (3) Secondary axes exhibited successively shorter total elongation, shorter duration of growth and slower daily growth rate from the topmost whorl downward. (4) Duration of elongation in the primary axis was greater than in secondaries in *P. strobus,* but in *P. resinosa,* duration of elongation in the upper 2-4 secondaries equalled that of the primary axes. All of these data covered the elongation exhibited during the 1942 season only. No other published work dealing with comparison of amount of elongation in primary and secondary axes of pines is known to the writers.

Since the appearance of the above paper, it has become of importance to know how generally the results obtained with respect to relation of total elongation in primary and secondary axes hold for the same tree over a period of years and in a larger number of trees.

METHODS

Length of "internodes on the primary axis and on three secondary axes for each year (false whorl) for each of 18 trees of red pine were measured with a meter stick. The trees used had been planted in 1940 as a reforestation project. The topmost 8 years of growth were used. Measurements were taken in early March 1952. The elongation used occurred during the years 1944-1951. Trees 1-9 were selected from the eastern marginal row of the planting and some of the lowermost 2-3 years of secondary branches show some degree of closure. Trees 10-18 were sufficiently separated that no closure was present. These latter trees were planted at the same time as trees 1-9,

FIG. 1

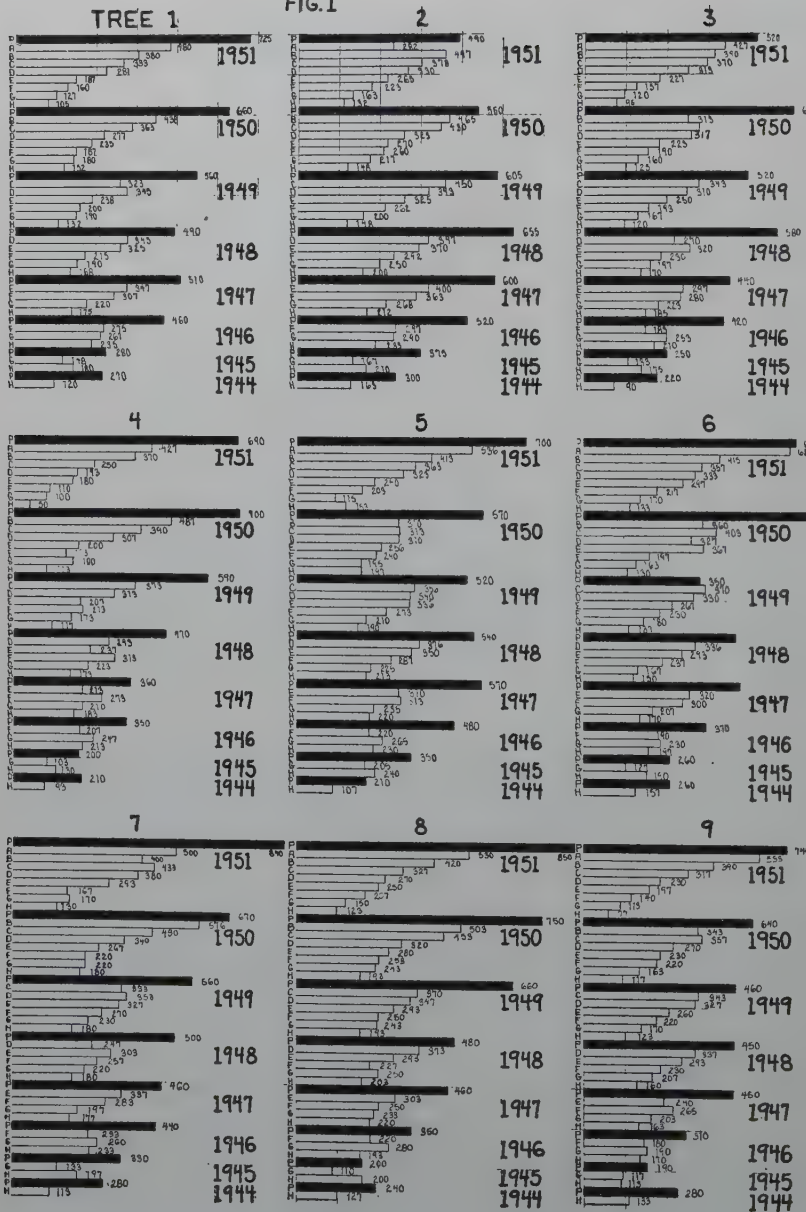
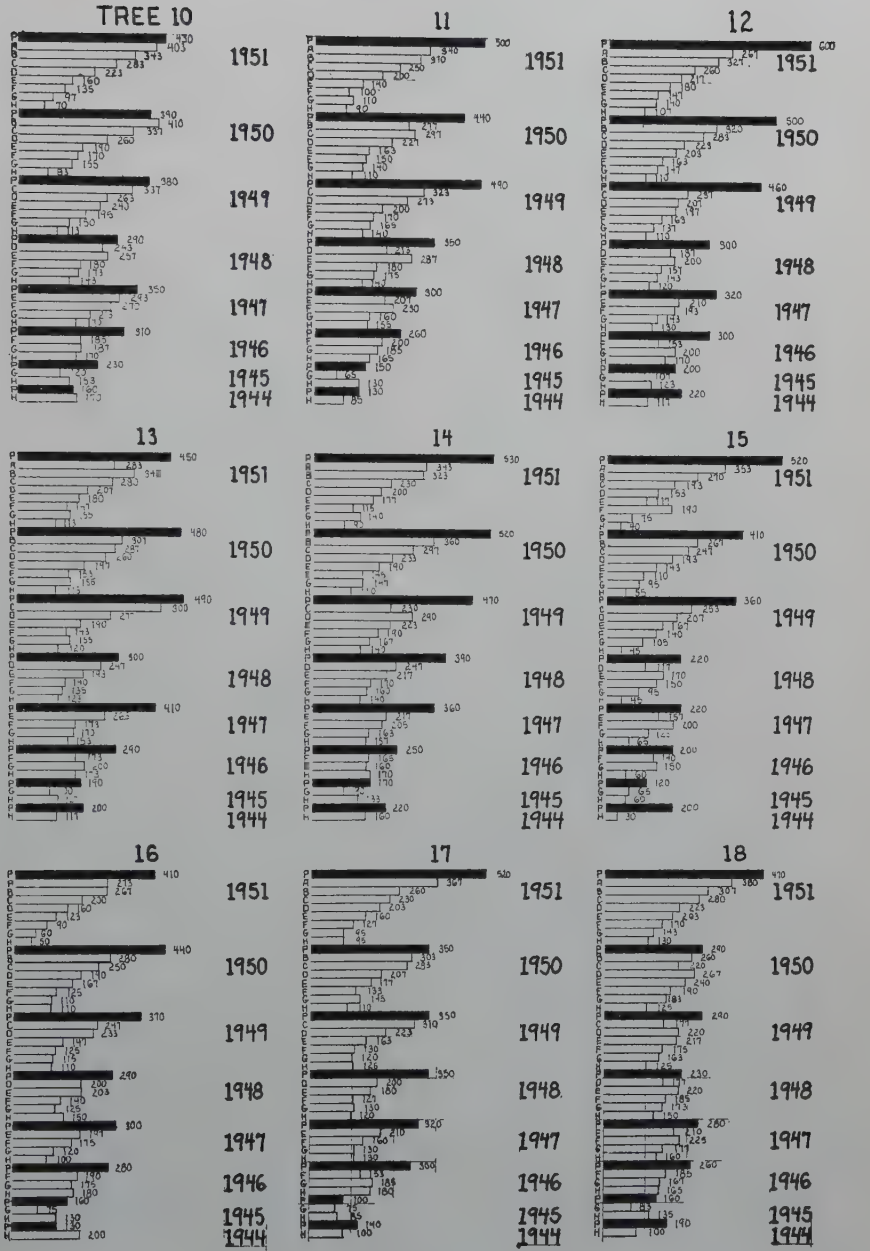


FIG. 2


TREE 10
11
12
13
14
15
16
17
18
1951
1950
1949
1948
1947
1946
1945
1944

but reference to their graphs will readily show that they had not reached the same height as that attained by trees 1-9. This difference is probably due to the fact that trees 10-18 were sufficiently near a forest stand of deciduous trees to be shaded for a period during the early part of the forenoon. No appreciable difference in relationship of elongation of primary to secondary branches between trees of the two groups is detectable.

RESULTS

Elongation of primary and secondary axes of each of the 18 trees is shown in figures 1-2. The topmost bar (P) in each group of bars represents the primary axis and successive secondary axes from the top downward are numbered A-H. The topmost group of bars (P, A-H) represents growth during 1951; the next lower group of bars (P, B-H) represents growth during 1950, etc. Obviously, secondary branch A will show in the graph for the year 1951 only; branch B will show for 1951 and 1950 only; branch C will show for 1951, 1950, and 1949 only; and so continuing down the tree; branch H will show all years, 1951-1944. The bars representing elongation in secondary branches were constructed from averages of 3 branches from each "whorl" except in a few cases where only 2 were present.

It will be noted that during every year of growth for all 18 of the trees, the primary axis showed greater elongation than the averages of the 3 secondaries which developed during the same year except for 4 of the 144 possible tree-growth years, viz. tree 6, during 1949; tree 10 in 1950 and in 1944; and tree 16, in 1944. It will also be noted that as a general rule the amount of elongation occurring during any particular year in secondary axes decreased successively on branches from the top downward. If we take tree 1 as an example (fig. 1), we find that during 1951 the primary axis elongated 725 mm; the average elongation of 3 of the topmost secondaries (A) was 480 mm; that of the next lower secondaries was 380 mm; and so on down the tree until the lowermost secondaries showed an average of only 105 mm. We note that branch H showed greater elongation for 1945 than did branch G above it.

If we consider all secondary branches for all years, there are 504 possibilities for a secondary branch to grow more or less during any particular year than the secondary above it. Of these 504 possibilities

FIG. 3

we find 76 cases where, during the same year, a branch grew more than the one above it. Exceptions thus occur in 15.1% of the possibilities.

Length of "internodes" on secondary branches has a strong tendency to be progressively greater from the younger toward the older portion of the same branch, i.e. from the tip of the branch toward the primary axis of the tree except for the oldest "internodes" of each branch. Figure 3 presents annual elongation for branches F, G, and H (the lowermost 3 branches) for each of the 18 trees. In the case of branch H (lowermost branch) every tree except trees 10 and 16 show length of median "internodes" to be greater than those at the tip of the branch and those next to the main axis of the tree. In 12 of the 18 trees, length of terminal "internode" of the branch is less than that of the oldest "internode" of the branch.

Branch G (next above the lowermost) shows much the same length-of-internode relation as branch H (fig. 3). In all 18 trees the longest "internodes" are median and the shortest are at the beginning and the end of each branch. In 8 trees the outermost (1951) 'internode" is the shortest; in the other 10 trees the innermost (1944) "internode" is the shortest.

Branch F (second above the lowermost) shows the median "internodes" generally longer than those at the tip and those nearest to the primary axis, but there are some exceptions, e.g. trees 13, 15, 16, 17 (fig. 3).

Among the secondary branches, there is a tendency for the longest "internode to be that "internode" which was formed during the year after initiation of the secondary, i.e. in the second year of its growth. The following table illustrates the point.

Branch	Initiated	Year of Longest Internode	Number of Trees
G	1945	1946 second year	14
		1947	1
		1948	1
		1949	1
		1950	1
F	1946	1946	1
		1947 second year	14
		1948	1
		1950	1
		One tie between '46 and '47	1
E	1947	1947	8
		1948 second year	7
		1949	1
		1950	2
D	1948	1948	5
		1949 second year	8
		1950	3
		1951	1
		Tie between '48 and '49	1
C	1949	1949	5
		1950 second year	8
		1951	4
		Tie between '49 and '50	1

It was noted above that there are 504 possibilities for a secondary to grow more in a particular year than the secondary above it. Within these 504, there are 126 possibilities for a secondary in its second year of growth to grow more than the secondary above it. Since 126 is 25% of 504, we might expect 25% of the secondaries which grow more than the secondary above them. to do so in their second year of growth.

Seventy-six instances where a secondary grew more than the one above were found. Of these, 52, or ca. 68%, of the longer "internodes" were on secondaries in their second year of growth. This is more than 2.5 times the percentage expected.

Amount of elongation for each year in the primary axes of the 18 trees is shown in figure 4. In general, the amount of elongation during each succeeding year has increased with increased age of the trees. Trees 2, 17, 18 present the greatest degree of divergence from this general trend. The character of the growth behavior of primary

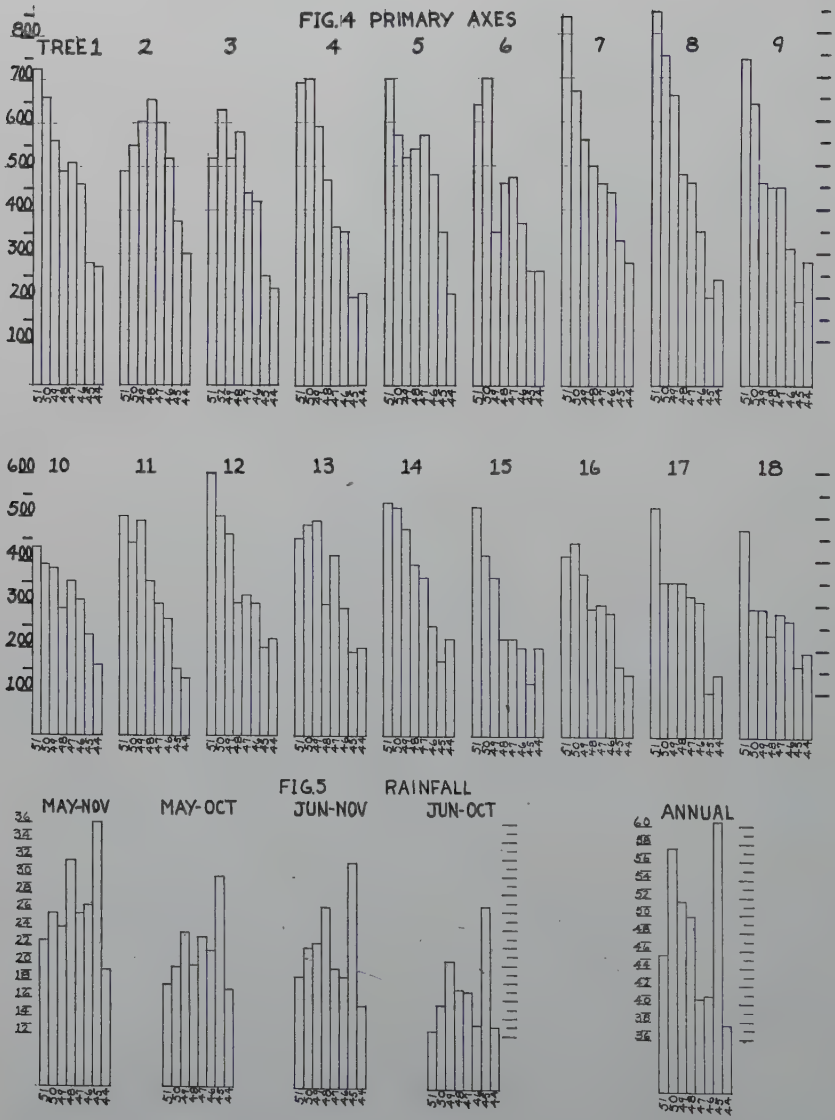


FIG.4 PRIMARY AXES
TREE 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
FIG.5 RAINFALL
MAY-NOV
MAY-OCT
JUN-NOV
JUN-OCT
ANNUAL

axes in these trees appears to justify the conclusion that they are too young to reveal obvious response to variations in the year-to-year environmental conditions which pertained at their present site during the 8 years for which data were taken. These trees were transplanted in April 1941, thus having had 11 growing seasons since being transplanted, the last 8 of which were used in this study. During these 8 years, height growth ranged from 2.170 to 4.095 meters. Trees 1-9 all showed total height growth for the 8-year period above 3m, while that for trees 10-18 was below 3m in all cases.

Miss Motley (2) found a striking correlation between annual axial growth of 100 trees of *P. strobus* and May-November rainfall of the preceding year. Elongation in four lower branches from each tree gave similar correlation. The composite curve for annual height growth in 50 trees of *P. resinosa* showed a general trend upward, though rate of increase over the preceding year was slackened in 1941 following a slump in May-November rainfall for 1940, and definite decrease in amount of elongation appeared in 1945 following a greater slump in May-November rainfall for 1944.

Graphs of rainfall for various month combinations are shown in figure 5. Comparison of growth graphs of primary axes with rainfall graphs shows no correlation. Even though the rainfall graphs show variation from year to year, the growth graphs show a general trend for each year to be greater than the preceding one. This lack of correlation is interpreted to be due partly to the fact that both annual and May-November rainfall was high for all years except 1944, which was the first year of measurements, and hence had no preceding year with which to be compared, and partly to the fact that the trees were still in the process of becoming established following transplanting. It should also be noted that Miss Motley (2) found that red pine does not show as marked correlation between elongation and rainfall of the previous storage season as is shown by white pine.

A significant correlation between amount of elongation and amount of rainfall during May-November of the preceding year is shown in branch H, the oldest secondary branches on these trees. Thirteen of the 18 trees studied formed the longest "internode" of branch H in 1946, the third year of growth. This is in disagreement with the general tendency to form longest "internode" in the second year of the life of the branch. Figure 5 shows the highest rainfall

for the years 1944-51 to have occurred in 1945, the storage season preceding 1946 growth. This, coupled with the fact that the lowest rainfall recorded occurred in 1944, should explain low growth in 1945 (the second year) and high growth in 1946 (the 3d year) for branch H.

High rainfall in 1945 would augment the tendency for branch G to grow more in 1946 (its second year), might influence the first year of branch F, and would not directly affect branches initiated after 1946.

SUMMARY AND CONCLUSIONS

1. Height growth in *P. resinosa* is greater in each particular year than the amount of elongation in any particular secondary branch for the same year. Exceptions were found in 4 out of 144 possibilities.

2. Amount of elongation in any particular year in secondary branches decreased successively on branches from the top of the tree downward. Exceptions were found in 15.1% of the possibilities.

3. Length of "internodes" on secondary branches has a strong tendency to be progressively greater from the younger toward the older portion of the same branch, i.e. from the tip of the branch toward the primary axis, except for oldest "internodes" of each branch.

4. In the present study, covering only the uppermost 8 years of growth in trees 11 years after transplanting, the amount of elongation in primary axes was greater each year than during the preceding year. No correlation in primary axes was discernible with rainfall. This is taken to indicate that the trees under study were too young and were still in the process of becoming established following transplanting.

5. The fact that rainfall was high for all years except the first year for which observations were taken probably helps to account for absence of any obvious growth-rainfall correlation.

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THE CULTIVATION OF ENDAMOEBA HISTOLYTICA AND THE IN-VITRO CHEMOTHERAPEUTIC TESTING OF AMEBACIDES[1]

By MAURICE E. CALLENDER

Endamoeba histolytica is the casual organism of the disease, amebiasis. Global distribution of high incidence makes this protozoan infection an enormous public health and economic problem. Cultural behavior and the ensuing search for effective chemotherapeutic agents has stimulated widespread interest in the parasitology and associated bacteriology of this organism.

A literature far too voluminous for presentation within the limitations necessary for the present paper has shown that it has not been possible to cultivate *E. histolytica* entirely free from microbial cells or their products. Apparently the concomitant microbial flora creates the physicochemical environment required by the amoeba for its continued well being. In 1949, Shaffer, Ryden, and Frye (21) published results of work which has been the closest approach to the goal of completely bacteria-free cultures. Their method, representing a few simplifications of an earlier one, used a medium containing a non-multiplying single species of an unidentified streptobacillus.

An adequate review of the literature dealing with the chemotherapy of *E. histolytica* is likewise beyond the limitations of the present paper. A few of the significant features may be briefly pointed out. Amebacidal properties of a number of antibioitcs have been studied. In the case of penicillin: Knoll and Howell (15) found no effect upon trophozoites. Streptomycin in concentrations of 10,000 u/ml was found to have no effect upon the cysts and some trophozoites were also unaffected, Belamuth and Wieboldt (3). Spingarn and Edelman (24) found that the addition of 1000-3000 u/ml of streptomycin prolonged the survival of cultures of *E. histolytica.*

[1] A portion of a thesis submitted in partial fulfillment of the requirements for the Master of Science degree in the Division of Graduate Instruction, Butler University. This paper has been greatly abbreviated for publication. The complete manuscript may be obtained on loan from the Butler University Library.

Using a combination of penicillin and streptomycin in equal unitage, Seneca, Henderson, and Harvey (19) published that 2000 u/ml kills amoeba in the first generation. However, Faust (6) found that a combination of 10,000 u of streptomycin and 5000 u of penicillin had no effect upon the amoeba. The present writer has found that a combination of 1000 u of penicillin and 2000 u of streptomycin per ml. can be used routinely in the isolation of trophozoites from their mixed bacterial flora.

Anderson et al. (1) found subtilin to be an effective amebacide in dilutions up to 1:400,000. Gould and Hansen (12) found that bacitracin, Polymixin B and streptomycin hydrochloride were lethal in "approximately 1/16 the minimal apparent amebicidal concentrations of the separate compounds when they were added together to the open or sealed cultures."

Falsenfeld et al. (7) claim, without publishing supporting data, that *E. histolytica* was inhibited in vitro by aureomycin, bacitracin and neomycin. Hewitt, Wallace, and White (13) have found aureomycin to be effective in dilutions as high as 1:100,000. Fuller and Faust (9) obtained good growth of *E. histolytica* in dilutions of aureomycin ranging from 1:300,000 to 1:1,000,000. Ishihara and Felsenfeld (14) found that neomycin inhibited growth in concentrations of 100 units/ml. Thompson et al. (26) found chloramphenicol to be effective as an amebacide in dilutions up to 1:3,496, but Smith et al. (23) found it effective only up to 1:1000 dilution and attributed the result to inhibition of associated bacterial flora. McCowen, Callender, and Lawlis (17) have found fumagillin, a new antibiotic, to be effective as an amebacide.

Emetine has given variable results when used as an amebacide, dilutions varying from 1:10,000 to 1:1,000,000 being effective (Fulton et al., 10; Dennis, Berberian, and Hansen, 5; Hewitt, Wallace and White, 13; Laidlaw, Dobell, and Bishop, 16; St. John, 25; Goodwin, Hoare and Sharp, 11). The last named workers also found cabarsone and chiniofon to be effective in dilutions of 1:10,000.

The present paper presents results from a study of several strains of *E. histolytica,* their cultivation on various media, and their exposure to antibiotics and known amebacides.

MATERIALS AND METHODS

Cultivation

This phase of the investigation was an attempt to establish strains of *Endamoeba histolytica* with various single species of bacteria and with various mixed bacterial flora. Three strains of *E. histolytica* have been used. These strains include the F-19, originally isolated by Dr. E. C. Faust, and NIH 200, obtained from Dr. C. W. Rees at the National Institute of Health. Nine species of bacteria were used in this study. All bacteria mentioned in this paper are named according to the 6th edition of Bergey's *Manual of Determinative Bacteriology.* Some of the bacteria were selected because they had been included in similar work by other investigators. Other species were selected because of their similarities to organisms in the first group; and still others were selected because of their widely differing characteristics from those of the first and second groups. Five media were utilized. These media included the Frye-Meleney (8) modification of the Boeck-Drbohlav (4) media, the Entamoeba medium of the Difco Laboratories, the Egg Infusion medium of Balamuth (2), the medium of Shaffer and Frye (20), and a medium consisting of nutrient agar slants overlayed with a serum saline mixture. Tables IV[2] through VIII[2] show the preparation of the various media.

The method used for experiments 1, 3, 4 and 5 was as follows: The media was inoculated with a 2 mm loop of bacterial organism obtained from a 48-hour culture grown on nutrient agar slants. The inoculated media was incubated for 24 hours at 37° C. After this period of incubation, sterile rice powder (Difco) was added and the media inoculated with 0.5 ml of a 72-hour culture of *E. histolytica* grown in the Shaffer-Frye (20) medium. The completely inoculated cultures were examined at 72 hours by taking a portion of the sediment containing rice powder and debris and examining under the microscope.

Experiment 2 differed in that the inoculation of *E. histolytica* was taken from the sixth culture of *E. histolytica-Bacillus cereus* from ex-

[2] Table numbers are those of the original thesis. Since their results are summarized in the published portion of the thesis, the tables are omitted from publication because of printing costs. They may be secured on loan from the Butler University Library.

periment 1. Experiment 6 was an attempt to establish the growth of *E. histolytica* upon a more simple medium, nutrient agar slants with an overlay of serum saline. Table X[2] lists the experiments with the various bacteria, amoebae and media used in each.

Chemotherapy

Five strains of *Endamoeba histolytica* have been used in the chemotherapy testing. These strains include the NRS strain obtained from Dr. J. G. Shaffer, Vanderbilt University; Luna, also obtained from Dr. Shaffer; NIH 103, obtained from Dr. Rees of the National Institute of Health; and the previously mentioned NIH 200 and F 19. The NRS and NI H103 have been used widely by many investigators seeking new amebacides. The NIH 200 is a strain highly pathogenic for laboratory animals and is being used as the infecting organism for *in-vivo* chemotherapy studies in rabbits, guinea pigs and rats. Strain F 19 has been utilized for the bulk of the work reported in this paper. The Luna strain is the most recently isolated of these five strains of amoebae. The isolation was accomplished by Dr. Shaffer at Vanderbilt University in February 1948. This was the first strain to be isolated from a human case of amoebiasis using the clear medium of Shaffer and Frye (20). Many parallel tests were run, using a compound against these several strains of amoebae. No appreciable difference was noticed in the behavior of the different strains. All of the strains employed were in regular association with mixed bacterial flora with the exception of strains grown and tested with the Shaffer-Frye (20) medium.

Test procedure I utilized a modified Boeck-Drbohlav medium, (tables IV[2] and IX[2]). The procedure of the test is as follows:

TEST PROCEDURE I

1. Add drug to overlay in tubes to make final desired dilution.
2. Add inoculum of 48-hour culture of *E. histolytica.*
3. Incubate 48 hours at 37° C.
4. Examine sediment for presence of amobae and subculture all negative tubes and faintly positive tubes.
5. Examine subcultures after 48 hours incubation at 37° C.
6. Record final results from the subcultures. (*Complete* absence of amobae in subculture tube is indicative of activity).

Test procedure II used the liquid Balmuth (2) Egg-yolk Infusion medium. See table VI[2] for preparation and composition of this medium. The procedure for this test is identical with procedure I.

Test procedure III also used the liquid Balamuth Egg-yolk Infusion medium. The details of this procedure follow.

TEST PROCEDURE III

1. Inoculate all tubes with 72-hour culture of *E. histolytica.*
2. Incubate 48 hours at 37° C.
3. Examine all tubes for presence of amoebae.
4. Add necessary amount of drug to tubes to give desired dilution.
5. Re-incubate 24 hours at 37° C.
6. Examine all tubes and make necessary sub-cultures.
7. Incubate subcultures for 48 hours at 37° C.
8. Examine subcultures and report.

Test procedure IV used the modified Shaffer-Frye medium described in table VIII[2]. The details are as follows:

TEST PROCEDURE IV

1. Place 2.0 ml of serum, saline, Penicillin G mixture in all tubes.
2. Place drug in first tube and make proper dilution to give desired drug dilution.
3. Add 2.0 ml of streptobacillus supernatant to each tube.
4. Add 1.0 ml of inoculum of *E. histolytica* to each tube.
5. Overlay tubes with sterile vaseline and incubate 48 hours at 37° C.
6. Examine each tube for presence of amoebae and make necessary subcultures.
7. After 48 hours incubation at 37° C, examine the subcultures.
8. Record final results.

Test procedure V also made use of the Shaffer-Frye medium.

TEST PROCEDURE V

1. Inoculate Shaffer-Frye media with *E. histolytica.*
2. Overlay with vaseline and incubate 48 hours at 37° C.
3. Examine each tube microscopically.
4. Add drug to each tube to give desired dilution.
5. Incubate 24 hours at 37° C.
6. Examine all tubes and make subcultures.
7. Examine subcultures after 48 hours at 37° C.

Test procedure I was used because it was the general method most often used by earlier workers. The medium used in this test has some serious faults. Prominent among these faults is the property of the egg slope to absorb certain chemicals. This can easily give erroneous results.

Test procedure II was the method most widely used in this paper. This method of testing utilizes a liquid medium which is low in protein and which also permits luxuriant growth of the amoebae.

Test procedure IV is the same method of testing as I and II, but utilizes an entirely different type of medium. This medium should give information as to the power of the amebacide to act directly upon the protozoan. This information is not clearly seen with the preceding test procedures.

Test procedures III and V, although using different media, are alike in that they test the power of the drug to destroy the organism as opposed to the power of inhibiting the growth of the organism.

In test procedures I, II and III, the inoculum of *E. histolytica* was in the range of 10,000-12,000 organism per ml of culture. This inoculum was obtained from 125 ml flasks containing the modified Boeck-Drbohlav media. A 72-hour culture of this was gently but well shaken and a representative sample counted in the manner described by Paulson and Morgenstern (18). Dilutions were then made to give the required number of organism per ml.

When utilizing the test procedures IV and V, smaller number of amoebae in the inoculum could be used. A number of amoebae amounting to 100 or 1,000 organisms per ml was used. This ten fold difference apparently led to no difference in results.

In all of the test procedures, many subcultures were made. All negative tubes were subcultured as were all faintly positive tubes. A number of heavily positive tubes were also subcultured for control purposes. Tubes designated as faintly positive contained less than 5 organisms per field when viewed through a microscope with 10 X objective and 10 X oculars. The effective dilution is that lowest dilution which is negative after the reading of the subcultures.

OBSERVATIONS AND RESULTS

Cultivation

Experiment 1: The inoculated cultures were examined at 72 hours by taking a portion of the sediment containing some rice powder and examining it under the microscope. This examination revealed motile organism only in the tube containing *Bacillus cereus*.

This tube was subcultured into fresh medium. Growth was maintained through a total of 17 subcultures at which time the cultures, still highly positive for *E. histolytica,* were discontinued.

Experiment 2: The growth of *E. histolytica* continued to be luxuriant with no apparent effects from the various bacterial mixtures. After 11 subcultures, with no demonstrable change in the numbers of *E. histolytica* present, the cultures were discontinued.

Experiment 3: Examination of 72-hour cultures failed to detect the presence of *E. histolytica* in any of the tubes.

Experiment 4: This was another repeat of experiment 1, and completely negative results were again obtained.

Experiment 5: No growth of *E. histolytica* was detected in any of the tubes inoculated.

Experiment 6: Both media used supported the growth of each of the amoebae-bacterial cultures through a series of 12 sub-cultures, at which time the various series were discontinued.

During this series of tests, it was noticed that if a drop of the positive cultures was placed on a glass slide, covered with a coverslip, ringed with vaseline or paraffin and reincubated at 37° C., the organism would survive and remain highly active and in large numbers for periods up to an additional 52 hours. This observation is of some use for classroom demonstration.

Chemotherapy

A total of 216 in-vitro chemotherapeutic tests are included in this report. Table XI[2] shows the complete tabulation of these tests. The results obtained using test procedure I correlated well with other investigators utilizing like methods. Emetine hydrochloride demonstrated activity at 1:20,000, Carbarsone oxide, 1:100,000 and Chloroquine, 1:500. Subtilin was the only antibiotic tested with this procedure, and no activity could be shown in a dilution of 1:500.

Test procedure II also correlated well with the observations of other investigators. This method of testing was applied to all of the drugs and antibiotics reported in this paper. Emetine hydrochloride demonstrated amebacidal activity in a dilution range of 1:100,000 to 1,500,000. Carbarsone oxide has amebicidal activity in the same

range as Emetine hydrochloride. Chiniofon was active in the range of 1:1,000 to 1:5,000 range. Carbarsone demonstrated no amebicidal activity in a dilution of 1,3,000. Chloroquine demonstrated amebicidal activity over a wide range, 1:500 to 1:5,000; but 84% of the tests performed showed activity at 1:1,000 or lower.

Among the antibiotics tested, Fumagillin was by far the most active substance tested. Dilutions as high as 1:262,144,000 completely inhibited the growth and reproduction of the amoebae. This antibiotic is especially interesting in that it possesses no anti-bacterial spectrum. Actidione demonstrated activity of a high order with a 1:10,000,000 dilution proving effective. Aureomycin was active at 1:64,000 dilution, with Terramycin showing activity at 1:32,000. Chloromycetin was active in a dilution of 1:4,000 while Bacitracin and Subtilin failed to demonstrate activity in a dilution of 1:500.

A modification of test procedure II by overlaying the medium with liquid vaseline was also used to test the activity of Fumagillin with identical results being obtained.

Test procedure III was used in the evaluation of Fumagillin. Here again, activity could be shown in a dilution of 1:131,072,000.

Test procedure IV was utilized to show the ability of the compounds to act upon the amoebae directly. The previous test procedure results are all difficult to evaluate because of the adsorption of the drug, interference of bacteria, and the action of the drug upon the bacteria. Emetine hydrochloride demonstrated activity in a range of 1:20,000 to 1:100,000. Carbarsone oxide continued to show activity in a range of 1:64,000 to 1:200,000. Chiniofon had activity at 1:2,000 while carbarsone was active at 1:8,000. Chloroquine failed to demonstrate activity at 1:500.

Fumagillin continued to demonstrate the highest amebicidal activity of any substance yet reported, with activity at 1:131,072,000. Actidione had activity at 1:20,000,000 to 1:50,000,000. Subtilin, which had failed to demonstrate activity in the earlier test procedures, now showed activity in the dilution of 1:20,000,000. The activity of Aureomycin decreased slightly to 1:32,000 while Terramycin dropped to 1:4,000.

Test procedure V was used only in the evaluation of Fumagillin. Activity was present in the dilutions of 1:131,072,000 to 1:262,144,000.

SUMMARY

Cultivation

A review of the cultivation of *E. histolytica* is given, and the possible role of bacteria in such cultivation is presented.

Attempts have been made to isolate trophozoites of *E. histolytica* with certain single species of bacteria. *Bacillus cereus* on one occasion supported the growth of the amoebae so that it could be cultivated with this single species. Other attempts to repeat this failed.

It has also been shown that the addition of *Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli* and *Micrococcus epidermidis,* either singly or in combination, to cultures of *Endamoeba histolytica* growing with *Bacillus cereus* failed to influence the growth of the amoebae.

Efforts to isolate the amoebae with pure cultures of *Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Micrococcus epidermidis, Bacillus circulans* and *Aerobacter aerogenes* also failed.

The cultivation of *Endamoeba histolytica* with a mixed bacterial flora and with organism "t" has been accomplished with the use of nutrient agar slants overlaid with a serum-saline mixture and with rice powder added.

Chemotherapy

A review of the *in-vitro* chemotherapy of *Endamoeba histolytica* is given. Several amebacides of clinical value and many of the newer antibiotics have been tested in various methods using five strains of *Endamoeba histolytica,* and the results are given. Of all the drugs and antibiotics tested, Fumagillin appears to be the most potent in every method. The antibiotic, Subtilin, presents a most interesting phenomena. This antibiotic, when tested with a mixed bacterial flora, demonstrates no amebicidal activity. When tested with a single species of bacteria, organism "t," activity is shown in a dilution of 1:400,000. Tests made with the Shaffer-Frye medium containing no actively multiplying bacteria, reveal activity in dilutions of

1:20,000,000. These last observations are in correlation with the direct microscopical examinations of Anderson, Villela, Hansen and Reed (1946). Table XII[2] compares the results reported in this paper with the results obtained by previous investigators.

CONCLUSIONS

1. *Endamoeba histolytica* can be isolated and grown with a pure culture of *Bacillus cereus*.

2. Addition of various other bacterial species failed to influence the growth of *E. histolytica* in association with *Bacillus cereus*.

3. It is possible to maintain cultures of *E. histolytica* growing both with a mixed bacterial flora and with a single species of bacteria upon a medium consisting of nutrient agar slants overlaid with a serum saline mixture with added rice starch.

4. Three methods of testing must be included for the complete evaluation of the compounds tested. One method should include a mixed bacterial flora to determine if the agent may be inactivated by such bacteria. Another method of testing should include no actively multiplying bacteria so that the direct effectiveness of the substance against the accompanying microbial organism may be ascertained. The third method of testing should be a method whereby the effectiveness of the substance to destroy as opposed to mere inhibition can be evaluated.

5. With the utilization of the fore-mentioned methods, one substance has consistently proved to be highly effective and should be thoroughly tested in cases of human amoebiasis. This substance is an antibiotic, Fumagillin.

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"CLAPOPHORA BALLS" COLLECTED IN STEUBEN COUNTY, INDIANA

By Fay Kenoyer Daily

On June 17, 1951, W. A. Daily and the author found some "Cladophora balls" washed out on shore and in the shallow water of Long Lake in the northwestern part of Steuben County, Indiana. These are hemispherical growths about one and one-half to two centimeters in diameter (Fig. 1A) and are formed from intertwined algal filaments of a felt-like nature (Fig. 1B).

The formation of "Cladophora balls" has been attributed to the result of wave action. Acton (1) in 1916 thought that the plant mass as it is rolled back and forth on the bottom of the lake assumes the spherical shape. Other plant tissues, pine needles, hair and the like have been found to form balls in a similar manner. Comparatively shallow lakes are usually the habitat necessary for "Cladophora ball" formation. Long Lake fits this category with an area of one hundred acres, an average depth of fifteen feet and a maximum of thirty-one feet.

The classification of the Cladophora found in these growths was made tentatively as *Cladophora* (formerly Aegagropila) *holsatica* Kütz. This was substantiated by Dr. Francis Drouet of the Chicago Natural History Museum, who pointed out, however, that Dr. Harry K. Phinney (2) included both *C. holsatica* and *C. Sauteri* in a broader classification under the name of *Cladophora aegagropila* (L.) Kütz.

The occurrence of "Cladophora balls" is common in Europe, but has been rarely reported for the United States. Apparently, there have been four records for this country. In his publication on the *Freshwater Cladophoraceae* in 1945, Phinney (2) reported *Cladophora aegagropila* (L.) Kütz. from Massachusetts and Wyoming. Dr. G. M. Smith (3) in his *Fresh-water Algae of the United States* reported "Cladophora balls" from Massachusetts and Minnesota. The latter is an error, this reference was to the collection made by Dr. L. A. Kenoyer in Michigan. A collection not cited by either Phinney or Smith was that reported by Dr. B. H. Smith (4) in *The Algae of Indiana* in 1932. *Cladophora Sauteri* was reported from

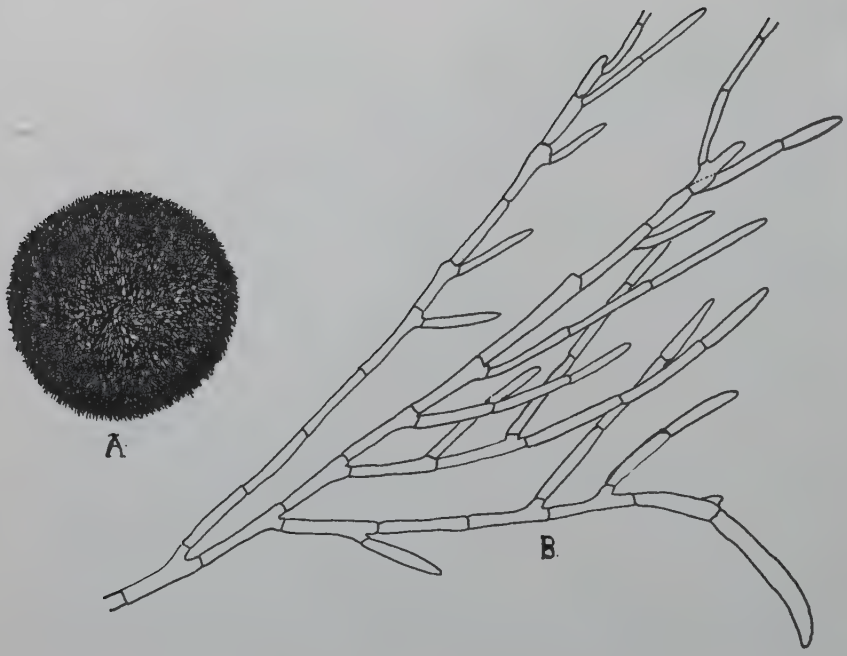

FIGURE 1
A. "Cladophora ball." B. Portion of a filament.

Kosciusko County, Indiana. A list of reported collections of *Cladophora aegagropila* (L.) Kütz should then include:

Collector	State Found	Date	Habit
F. S. Collins	Massachusetts	Sept., 1895	Washed ashore
W. G. Solheim	Wyoming	July, 1937	Floating
L. A. Kenoyer	Michigan	Aug., 1925	Shallow water along shore
B. H. Smith	Indiana	June, 1931	?

Portions of all reported specimens except the collection made by B. H. Smith have been examined. They may be found at the Chicago Natural History Museum. Collection 293 B made by W. A. Daily and the author may be found there also as well as in the herbarium at Butler University. Dr. Kenoyer's collection may be found at Butler University also.

The author is indebted to Dr. Kenoyer for stimulating an interest in this curious alga and for cooperating by sending a copy of his paper presented before the Michigan Academy of Science in 1939 in which he reported his collection of *Cladophora holsatica* Kütz. at Millcoquins Lake near Naubinway, Mackinac County, Michigan in 1925.

It will be interesting to note further occurrence of this alga.

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THE PIONEER PERIOD IN THE STUDY OF INDIANA VASCULAR FLORA

By Ray C. Friesner

The earliest scientific work on Indiana vascular flora was done by non-resident travelling naturalists. Andre Michaux, born in Satory near Versailles, France, March 7, 1746, spent ten years in North America, 1786-1796, during which time he travelled from Hudson's Bay to Florida and from the Atlantic to the Mississippi River, and collected many species of plants. This collection contained about 20 species taken from Indiana on a trip across the state from Louisville to Vincennes in August 1795 (12, 24). Four days were required for the trip of 125 miles. In commenting on this trip he writes, "Of all the journeys I have made in North America in the past ten years, this is one of the most difficult owing to the quantity of trees overturned by storms, to the thick brushwood through which one is obliged to pass; to the number of Flies by which one is devoured, etc." This total collection formed the basis for the first general flora of North America. The publication appeared in 1803 following his death in Madegascar in 1802.

His son, Francois Andre Michaux, travelled some 2000 miles on a trip through the Ohio valley in 1802. Observations on plants and agriculture were recorded briefly in his "Travels," but his major work, completed after further travels (2), was his "North American Sylva."

David Thomas, a civil engineer by profession, (born June 5, 1776, in Montgomery County, Pennsylvania), journeyed along the Ohio River from eastern Indiana and ultimately reached Vincennes in 1816. In the account of his travels he records 95 species of plants taken from Indiana (23). The following quotation makes an interesting reference to the situation regarding floods during that early period. "He (his host) pointed to a mark on the wall, about four feet above the first floor, and observed that the river had been there; and that they had taken refuge in the neighboring hills. It is said that the difference between high- and low-water mark sometimes equals 60 feet perpendicular, and our observations tend to give credence to this statement."

A few other quotations will give the present-day botanist a vivid picture of the character of the vegetation. "The rank vegetation of the river flats crowded so close as sometimes to brush both sides of us as we rode along and indeed everything conspired to remind us being in a new country. After a traverse of three or four miles we came to Rising Sun. This village of 40 or 50 houses is built on an easy slope that fronts the Ohio.

"On leaving, we ascended the hills, the soil of which is very fertile, and the vegetation uncommonly fine. We gazed at the majestic beech of this country, three feet in diameter, with branches of great size; . . . we had seen the honey locust, the black walnut and the horse chestnut (sweet buckeye) of equal magnitude; . . . and here we saw, with surprise, the black locust almost a rival in stature, with grape vines, like cables hanging from the tops of the trees in every direction.

"Chestnut grows near the base (of the knobs) and chestnut oak on the peaks; but as we leave these, and advance westward where the soil is less exposed to the wasting action of winds and rains, the timber becomes nearly as thrifty as on the plains below; and pawpaw and spice-wood, as usual constitute the principal underbrush."

Sugar maple and beech are mentioned often and he writes of the "open oak woods" passing "into an extensive plain or prairie. Here such are called barrens, but improperly, for the soil is very fertile." Apparently the term "barren" was applied in these early days to any treeless area surrounded by forest. He speculates on the cause of the prairie: "To me it is evident that the immediate causes of these wastes are fire and inundation; but the predisponent cause is either an impenetrable hardpan, or a level rock . . . the same rock extending under drier parts confines the roots and intercepts the supply of moisture that subsoils generally contribute. The trees thus stunted admit amongst them a luxurient herbage." The cork elm was given the scientific name *Ulmus thomasi* in his honor by Sargent (19).

Constantine Samuel Refinesque, born October 22, 1793, at Galata, the European residential district of Constantinople (1) was the earliest resident botanist west of the Alleghanies. During the 25-year period from 1815 to 1840, he travelled on foot through much of the central Mississippi valley and the states eastward to the Atlantic

seaboard, collecting, studying, and writing about the plants, animals, and other natural history materials of the area. He spent some of this time at the New Harmony settlement and mentions meeting Dr. Miller (Dr. Christoph Miller or Mueller), who gave him "some fine plants" and with whom he "went . . . to herborize in the meadows," (14).

No doubt he made numerous trips into the Ohio valley portions of Indiana during his professorship at Transylvania University. Of a total of 1235 Rafinesquian names (11) proposed for 634 taxonomic entities occurring in Indiana, he specifically cites 21 as being based on specimens from Indiana, but includes Indiana within the range of many others. Of the 21 Rafinesquian names based upon specimens from Indiana, only one, viz. *Oenothera pilosella* Raf. is now in use. Of the remaining 20 names, 6 (*Onoclea dentata* Raf., *Rosa dasistema* Raf., *R. acuminata* Raf., *Dodecathion augustifolium* Raf., *Scutellaria villosa* Raf., and *Lophactis unifolia* Raf.) are of unknown identity. The remaining 14 are invalid names. Of the total of 1235 names given by Rafinesque to 634 entities which occur in Indiana, only 33 or 2.66% are now considered to be valid names.

The genius, untiring energy, but careless, erratic, and contentious character of this pioneer in midwestern natural science is only now being appreciated, so great were the eccentricities of his behavior and his personality. Peattie (13) says of him, "Amongst all the naturalists who ever worked on the American continent, Rafinesque is the only one who might clearly be called a Titan. He had a voracious appetite for discovery, an energy that would have carried him triumphantly through the career of a Humbolt, a Darwin, a Linnaeus, all in one. But he had no mental discipline, no capacity to see any subject through to a conclusion, no respect for the work of others, no care for how he wounded feelings or made enemies, and absolutely no sense of humor."

The attitude of his contemporaries and those following him for several decades after his death (1840) is well expressed by the following quotation from a letter written by the botanist, George Engelmann, to the physician and botanist, Charles W. Short, under date of June 12, 1855 (4). "Your offer of Rafinesque's writings I thankfully accept; though I believe with you that the trouble of sifting the little good wheat from the mass of chaff is vastly greater than to go to work anew without paying attention to him. . . . Agassiz, however,

lately has paid more attention to his labors about fishes, and seems to consider him quite sharp-sighted, and ahead of his contemporaries."

Merrill (11) says of Rafinesque, "Evidence adequately warrants the conclusion that Rafinesque was a genius. He possessed a versatility of kaleidoscopic range. His work was frequently said to be careless, incorrect, and unreliable. His ceaseless compulsive mental activity, rapidity of execution and magnitude of output—representations of his character organization—adequately account for whatever lack of integrity his work possessed. . . . Anyone who attempts to evaluate Rafinesque's botanical work on the basis of a thorough examination of his numerous publications can reach only one conclusion. Briefly, this is that it is regrettable that he published so much and so poorly. . . . He was erratic, nonconformic, contentious, positive of the correctness of his views, highly critical of his contemporaries and some of his predecessors. . . . Clearly in some respects he was ahead of his time. His judgment was faulty, he being uncontrolled and uncontrollable. Some of his contemporaries and successors even considered him insane."

Coulter (5) records a visit by Thomas Nuttall to the Ohio River area of Indiana in 1818, a few years before he became professor of Natural History and curator of the botanical garden at Harvard University. While Nuttall is credited as the name authority of a great many species which occur in Indiana, there is no evidence that his descriptions are based upon any Indiana specimens.

Alexander Philip Maximilian, Prince of Neuweid, travelled in the United States in 1832-34. He began his journey at Boston and continued to New York, Philadelphia, and Pittsburgh, thence down the Ohio to the Wabash. He landed at Mount Vernon (Indiana) and proceeded by road to New Harmony, arriving October 19, where he spent the winter of 1832-33 with C. A. Lesueur, Thomas Say, and other naturalists. In the spring he continued his journey via steamer down the Wabash and the Ohio to the Mississippi, thence to the mouth of the Missouri which he ascended to the Rocky Mountain region.

Returning by a different route, he arrived in New Harmony in May, 1833 and continued his study of the local flora. He left New Harmony on June 9, 1833, accompanied by Lesueur, and proceeded via wagon road through Owensville, Princeton, and Vincennes, thence

eastward across Indiana and northward to Lake Erie, Niagara, and back to Boston. The results of his study were published in his "Reise durch innere Nord-Amerika" (10) a large quarto volume with numerous wood cuts and an atlas of magnificent folio plates.

First mention of plants of Indiana occurs in his notes for October 16, 1832. Some delay was experienced during completion of necessary repairs to the boat on which he was travelling and "We took advantage of the delay to walk in the woods, for the first time in the state (Indiana). The bank was about 50 feet high and steep; the upper part of this declivity was grown with Datura, the seeds of which were not ripe, but very few of the bright violet flowers were to be seen. The handsome blue-flowering *Eupatorium coelestinum* and the *Lobelia siphilitica* bore their flowers between the thorn apples. Upon the bank was a magnificent forest of tall heavy beeches, maples, oaks, walnuts, etc., in which some plantations of maize with their block houses were already to be found. As underwood, the papaw tree grew here everywhere; in the edge of the forest the yellow-flowering *Cassia marilandica* with ripe seeds. Here stood colossal trunks which three or four men could not have spanned."

Of the vegetation about New Harmony, he writes, "Lofty forests rise round about, in which the settlers have here and there established their fields. The forests which they inhabit are very extensive, their ground very fertile. Nature is here far more vigorous and luxurient than eastward of the Alleghanies." Plants mentioned include mistletoe, cross vine, sycamores over 41 feet in circumference, lofty tulip trees, maples of immense height, nine species of oaks, walnut, wild cherry, eight species of hickory, beech, elms, hackberry, sweet gum, coffee-nut, catalpa, black gum, ash, linden, buckeye. In all, 58 species of trees and 13 species of shrubs are listed, besides the more casual mention of herbaceous species. This is the earliest published list of trees and shrubs in Indiana.

In 1835, John L. Riddell published the first comprehensive list of plants (17) of the general area of which Indiana is a part. His synopsis contained over 1800 species and included many common in Indiana. Since he did not retain verifying specimens upon which his publication was based, it is not certain that any of the particular specimens used for his data actually came from Indiana. While he taught botany (natural history) for a short period in the Worthington

(Ohio) academy (22), his interests were primarily in medicine, of which he became a professor in the Medical College of Louisiana. Nevertheless, his synopsis and subsequent supplementary catalogue (18) had a unifying and organizing influence in the pioneer taxonomic work of the Northwest Territory.

Dr. Charles W. Short, a physician of Lexington, Kentucky, mentions, in his Fourth Supplementary Catalogue published in 1840, *Psoralea congesta,* a new species lately discovered by Dr. Clapp and Mr. Jones, of New Albany, on islands of the Ohio River, near that place (21). *Solidago shortii* from near Jeffersonville was named by Riddell in honor of Dr. Short.

Alphonse Wood published the second edition of his "Class Book of Botany" (25) in 1847, in which he described *Sabatia concinna* Wood as a new species from Indiana: "Dry, grassy prairies, Ia.! abundant." In the third edition of this manual (1851) (26), he described *Gerardia Skinneriana* Wood as a new species from the farm of Dr. A. G. Skinner in Green County, Indiana: "Barrens, Ia.!" Regarding this latter species, he appends the following note at the end of the technical description, "I detected this delicate species in July, 1846, in Green Co., Ia., on land belonging to Dr. A. G. Skinner, whose zeal in botanical pursuits deserves more than this slight notice. It does not turn black in drying."

It should be noted from the above paragraph that the abbreviation "Ia." was used by some, but not all, of the earlier writers in referring to Indiana. Proof that Indiana, and not Iowa, was meant, is found in the way the following three persons, commenting on Wood's book, signed their names: "Henry Ward Beecher, Indianapolis, Ia., Editor of Western Farmer and Gardner;" "Dr. John T. Plummer, Richmond, Ind.;" and "Dr. A. G. Skinner, Greene Co., Indiana."

Dr. Asahel Clapp (1792-1862) is the earliest Hoosier resident to follow the study of systematic botany. Locating in New Albany as a physician in 1817, he quickly made himself familiar with the plants of that area. In May, 1852, he presented his paper on "Medicinal Plants of the United States," (3) before the Philadelphia meeting of the American Medical Association.

Dr. Clapp's copy of Riddell's "Synopsis of the Flora of the Western States" is an interleaved copy and in it he has indicated the plants

which he collected. He states that he has added 100 species not recorded by Riddell. His copy of Gray's "Manual of the Botany of the Northern United States," (first edition of Gray's Manual), is also marked, and from these two, together with the list of medicinal plants presented in his paper mentioned above, it has been possible to reconstruct a list of plants collected by him in Floyd County and vicinity. The list totals 930 species. The Wabash College herbarium contains 184 specimens; Purdue University 18; and Chicago Natural History Museum, 17 specimens collected by Dr. Clapp. His list of medicinal plants collected in the vicinity of New Albany contains 292 species.

In 1854, Increase A. Lapham, engineer, botanist, paleontologist, geologist, and expert map maker, published (9) a list of over 800 species of grasses of Wisconsin and adjacent states, including Indiana. Apparently this is the earliest comprehensive publication devoted exclusively to the grasses of the Northwest area.

Rufus Haymond, in his geological survey of Franklin County (8), presented a list of the principal timber trees of that county, prepared during the summer and fall of 1869. The list contains 33 species with white oak as the most frequent, and beech a close second.

He lists both "white" (*F. sylvestris* Michx.) and "red" (*F. ferruginea* Ait.) beech. Wood, (26) in his "Class Book of Botany" 3 ed. (1851) writes, "The *Red Beech* is now regarded as only a variety; with wood softer, and of more easy cleavage, and perhaps a slight difference in foliage." All other species, though listed under scientific names in use at that time, are identifiable with names in use in our present time. One Indiana specimen bearing his name as collector is deposited in the herbarium of the Chicago Natural History Museum (26).

R. H. Fisher, of Arba, Randolph County, writes in the American Naturalist, 1870, of having in his collection a specimen of *Trillium sessile* with flower parts in fours and one of *T. recurvatum* with parts in twos. These were collected along the Salamonie River (6).

Thus ended what may be spoken of as the pioneer period in the study of Indiana vascular plants; a period of 75 years, during which this study was carried on by three types of naturalists: (1) Travellers who passed through the state or made only brief stays, collecting and

observing, but moving on to other centers for study and writing of their results. Such workers are illustrated by Andre Michaux (1795), Francois Andre Michaux (1802, ff.), David Thomas (1816), Thomas Nuttall (1818), and Alexander Philip Maximilian (1832-33). (2) Professional botanists of the general area but residing outside the state, e.g. Constantine Samuel Rafinesque (1793-1840), who was Professor of Natural History at Transylvania University, Lexington, Kentucky, and who no doubt made many collecting trips into Indiana; John L. Riddell, whose botanical work was done in Ohio, but who undoubtedly had many Indiana specimens for study, including *Solidago shortii,* and who did much to unify and stabilize taxonomy of that time. Alphonse Wood, whose Class Book of Botany (1847 and 1851) was widely used in the later years of the pioneer period and the earlier years of the middle period, described at least two new species from specimens first collected in Indiana. (3) Men of other professions who did much as side lines of interest to advance our knowledge of Indiana flora, e.g. Dr. Charles W. Short, physician of Lexington, Kentucky (1835), and Dr. Asahel Clapp, physician of New Albany, Indiana (1852).

The publication of "Manual of Botany of Jefferson County" by A. H. Young (27) in 1871, and the activity of the Hanover group, marks the beginning of the Middle Period (1871-1900).

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. Charles C. Deam, whose collection of original materials made this study possible, and whose inspiration and encouragement furnished the motivation for this first part of a larger project intended to cover the history of Indiana taxonomy from its beginnings to the present date.

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THE EFFECT OF CERTAIN BACTERIA UPON THE GROWTH OF TRICHOMONAS VAGINALIS IN VITRO[1]

By JOHN F. LAWLIS, JR.

Trichomonas vaginalis was discovered by Donne in 1837, and for it he established the genus Trichomonas. It is, therefore, the type species of the genus. The organism is a flagellate protozoon. Measurements vary according to the age of the organism, from 18 x 6 microns to 26 x 16 microns. Usually there are four anterior flagella and an axostyle protruding from the posterior end. An undulating membrane attached to the anterior end reaches back approximately one-half the length of the organism (Wenyon, 24). Recent authorities who have carefully studied the trichomonads parasitic in man, believe that *T. buccalis* and *T. hominis* are identical with this species (Wenyon, 24; Dobell, 5).

T. vaginalis occurs in the vaginal secretions of women when the secretions are acid, instead of alkaline in reaction, and even though a diseased condition of the mucous membrane of the vagina be present, this parasite is not found if the secretion is alkaline. It has also been found in the urine of both men and women, and Stuhler (20) states that in the examination of 32,000 prostatic secretions at the Mayo Clinic, *T. vaginalis* was present in 16 cases.

HISTORY

For 80 years after the human vaginal trichomonad was first described, little attention was given to its possible pathogenicity. Hoehna (9) developed the concept that this protozoon is a pathogen and introduced the term "trichomonaskalpitis" to define this activity. During the succeeding years, opinion has been divided as to whether the organism actually is the causative agent of the irritation with which it is associated or whether it is merely living symbiotically with bacteria to produce the pathology.

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[1] A portion of a thesis submitted in partial fulfillment of the requirements for the Master of Science degree in the Division of Graduate Instruction, Butler University. The Parasitological Laboratory, Eli Lilly Company.

Hibbert et al. (8) concluded that a bacterial organism, *Streptococcus subacidus*, which he isolated from a number of *T. vaginalis* cases, was the actual causative agent in vaginitis. Trussell and Plass (21) found the typical syndrome of *Trichomonas vaginitis* in five of nine trichomonad-free women whom they were able to infect with the organism in pure culture. They concluded that their failure to identify *S. subacidus* in the successful cases would indicate that this organism was not essential to the development of vaginal irritation. Attempts to infect various animals with *T. vaginalis* by intra-peritoneal, vaginal, intra-venous, and intra-cranial inoculations failed to produce lesions of any type. Vanni et al. (22) presented histological evidence of invasion of the cells of Lieberkuhn as well as the mucosa by *T. hominis* (considered by many workers to be physiologically and morphologically the same organism as *T. vaginalis*). Schnitzer et al. (15) submitted evidence that at least one strain of *T. vaginalis* is pathogenic for mice and may produce characteristic lesions following the injection of bacteria-free cultures by the intra-abdominal, intra-muscular and subcutaneous routes.

T. vaginalis was first obtained in pure culture by Trussell and Plass (21). These authors found that one of their stock cultures had overgrown its associated bacteria and had thus purified itself. Unfortunately, they had not been able to determine the cause of this peculiar condition and, consequently, were unable to duplicate or repeat the phenomenon with other strains.

Adler (1) obtained bacteria-free cultures of *T. vaginalis* by use of penicillin G. This antibiotic, at low levels, did not have a deleterious effect upon the growth of *T. vaginalis*. It was found that the bacterial contaminants tend to adhere to the trichomonads, thereby complicating usual pure-culture isolation technique, but the use of the antibiotics eliminated this complication. Johnson et al. (11) were able to isolate *T. vaginalis* in pure culture by the concurrent introduction of penicillin G and vaginal exudate into the isolation medium.

Shaffer and Frye (16) established the growth of pure cultures of *T. vaginalis* in a medium composed of (a) a "preconditioned" thioglycollate medium, (b) a bacteria-free filtrate of a culture of the bacterial complex associated with the NRS strain of *Endamoeba histolytica*, (c) normal horse serum, (d) normal saline (0.85 per cent NaCl), and (e) penicillin G, 100 units per ml of medium. The thioglycollate

was preconditioned with a Gram negative streptobacillus culture. After incubation at 37° C. for 18-24 hours, this culture was centrifuged at 2,000 r.p.m. for 45 minutes. The supernatant fluid was withdrawn and constituted the preconditioned thioglycollate. This type of cultivation in effect gave a bacteria-free culture of *T. vaginalis* since the penicillin was in sufficient concentration to inhibit bacterial growth effectively.

Sprince et al. (19) found an unidentified factor, essential for sustained growth of *T. vaginalis,* in a lipocaic fraction of prancreas. Further research on this fraction may lead to a completely synthetic medium. Lash (13) used a simplified casein hydrolysate-serum medium consisting of Casamino acids, maltose, dextrose, sodium lactate and physiological salts in distilled water for the cultivation of *T. vaginalis.* The medium is rendered complete by the addition of 20% beef blood serum and buffers. The method used to purify cultures is not given.

Chemotherapy of *T. vaginalis* vaginitis has varied according to the practitioner's concept of the causative agent. It would seem that Adler et al. (1) did the primary work with antibiotics when they successfully isolated the protozoon in pure culture by the use of 250 units penicillin G per culture. Although they apparently did not intend this as an objective, they did prove that penicillin per se had little or no effect upon *T. vaginalis* at this particular dosage level. Johnson et al. (11) further threw light on this subject when they obtained *T. vaginalis* in pure culture from vaginal swabs by using 5,000-10,000 units of penicillin at 60 hours exposure. The trichomonads were not affected at this high concentration of penicillin.

Johnson et al. (10) attempted in vivo treatment of *T. vaginalis* with tyrothricin using suppositories as a vehicle. Gramicidin and tyrocidin were isolated from *Bacillus brevis* by Dubos (6), and the combination of the two makes up tyrothricin, which is a surface-acting antibiotic. However, the ineffectiveness of the compound, coupled with its high toxicity, yielded very unfavorable results. Waksman et al. (23) found streptocin, an antibiotic isolated from the mycelium of *Streptomyces griseus,* to be active against *T. vaginalis* in vitro at 1 :20,000. They used 250 units per ml of each of streptomycin and penicillin to prevent bacterial contamination.

Greenblatt (7) determined the in vitro efficiacy of streptomycin, aureomycin, tyrothricin, penicillin, penicillin (procaine), chloromycetin, and bacitracin against *T. vaginalis*. Of these antibiotics, penicillin, penicillin (procaine) and bacitracin were of little value; whereas, aureomycin was probably the most effective, with tyrothricin second, chloromycetin third, and streptomycin fourth.

Sicard et al. (18) reported that conossine, an alkaloid of *Halorrhina floribunda*, whose amebacidal properties are well known, was effective in 34 out of 48 patients treated for *T. vaginalis*. Kleeberg et al. (12) observed a side effect of aureomycin increasing the number of *T. intestinalis hominis* present in patients through an alteration of the intestinal flora.

Shaffer and Biegeleisen (17) showed that chloromycetin and aureomycin, at a rather high concentration, are effective trichomonacides. Chloromycetin even in the highest concentration used, which was 1.47 mg per ml, was probably not trichomonacidal, but it did markedly inhibit growth and multiplication in concentrations as low as 0.37 mg per ml. Aureomycin completely inhibited *T. hominis* at a concentration of 0.059 mg per ml. The findings in this study support the findings of Greenblatt (7).

It is the purpose of this paper to determine the effect of certain more common bacteria upon the growth of *T. vaginalis* in vitro and also to determine the effect of some of their filtrates when using a medium which is routinely used as a cultural media for other parasitic organisms. It is felt that a more critical survey could have been made if the bacterial organisms usually found in the vaginal tract had been used; however, such organisms were not available to the writer. Considering the complete absence of reports in the literature on bacterial effects upon *T. vaginalis* in vitro, it was deemed a worthwhile study.

MATERIAL AND METHODS

Seventeen different bacterial organisms were used in this study. Four were obtained from the Eli Lilly Laboratories, Department of Antibiotic Research, two from the Department of Parasitological Research of the Eli Lilly Research Laboratories, and the remaining eleven from the Department of Botany, Butler University. Since

each organism was identified by the source, the writer did not attempt to substantiate the identification.

The following were obtained from the Lilly Laboratories: *Aerobactor aerogenes, Bacillus brevis, B. circulans, B. polymxa,* Organism "t." and a Streptobacillus; the following were obtained from Butler University: *B. megatherium, B. subtilis, B. subtilis* (morphotype) *panis, Escherichia coli, E. coli* var. *neopolitana, E. paragruenthali, Klebsiella capsulata, Micrococcus epidermidis, Proteus vulgaris, Pseudomonas aeruginosa,* and *Sarcina lutea.*

For a stock culture, each organism was cultured on nutrient agar (formula 1) slants overlayed with sterile mineral oil, which prevents dehydration, and stored at 4° C. Sub-cultures were routinely made to ascertain the condition of the bacteria. A Gram stain was done at this time to determine presence of organisms.

FORMULA 1. NUTRIENT AGAR COMPOSITION

Bacto-Beef Extract	3.0g
Bacto-Peptone	5.0g
Sodium Chloride	8.0g
Bacto Agar	15.0g
Distilled Water	1000 ml

The particular strain of *T. vaginalis* used in these experiments was isolated from a patient at the Indiana University Medical Center in 1949. The organism was routinely cultured on Shaffer and Frye (16) bacteria-free medium (formula 2). This method was used for stock cultures.

FORMULA 2. SHAFFER-FRYE MEDIUM

Pre-conditioned thioglycollate	2.5 ml
Bacterial complex filtrate	1.5 ml
Saline	0.25 ml
Serum	0.5 ml
Penicillin	500 units

First, it had to be determined which of available media was the most productive for *T. vaginalis* as well as the bacteria. The fact that *T. vaginalis* would grow equally well in either Boeck-Drbohlav (4) whole egg medium or Balamuths (2) egg-infusion medium upon the addition of 10% horse serum had been determined previously by the writer. The consideration to be made at this point was which medium

would be most effectively used as a filtrate and as a testing medium. It was felt by the writer and has been supported by other workers that a diphasic medium such as Boeck-Drbohlav whole egg may tend to absorb or adsorb the active principle in an antibiotic substance. Therefore, Balamuths egg yolk infusion with 10% horse serum (see table I for preparation and ingredients) was selected as the medium for studies with bacteria.

The bacteria were taken from the stock culture of a nutrient-agar slant with a heat-sterilized 2mm. platinum loop. These transfers were carried out in a sterility tested cage in order to diminish the possibility of contamination. The loop of bacteria thus obtained was then inoculated into a tube of egg-infusion medium which had been incubated at 37° C. for 24 hours previous to this inoculation of bacteria, to insure sterility. For each strain of bacteria, two tubes were inoculated by this method. In all, three such sets of experiments, or 102 tubes of media, were inoculated with bacteria to determine growth under normal conditions. These cultures were placed in an incubator at 37° C.[1] and examined macroscopically and microscopically (Gram stain) to determine growth.

One ml of a 72-hour stock culture of *T. vaginalis* was pipetted into a tube of egg-infusion medium and incubated at 37° C. for 168 hours. At 24-hour intervals, these tubes were removed and examined microscopically for trichomonads. Twenty such cultures were made, and a normal growth curve was established.

Experiment 1. Culture of bacteria with *T. vaginalis*.

In order to facilitate large scale testing, which was necessary for a critical test, large amounts of trichomonads had to be available for inoculation. A technique used for large yields of *Endamoeba histolytica* was considered applicable to this situation. Test tubes, 26 x 165 mm in size, with 4 times the stock amount of Shaffer-Frye medium, were prepared. By seeding one large test tube from one small tube, 4 times the amount of inoculum was made available from a single source. In doing this, the problem of pooling the inoculum was eliminated; therefore, the chances of contamination were decreased. The growth in the large test tubes after 72 hours incubation

[1] 37° C. was selected because the protozoon grows best at this temperature.

at 37° C. was very luxuriant, so this technique was adopted as a source of inoculum for all tests. Egg-infusion medium was inoculated with bacteria from stock cultures and allowed to incubate at 37° C. for 48 hours. These tubes served as another source of inoculum.

Twenty-four hours prior to a scheduled test run, the tubes of egg-infusion media to be used were placed in the incubator, so as to condition them properly as to temperature, and also to observe any contamination. One ml of the trichomonad inoculum was pipetted into a test tube of medium, and then one ml of bacterial suspension was added. Two such tubes were made for each organism, and an additional two tubes were inoculated with *T. vaginalis* and served as controls. After the tubes had been inoculated, the cultures were placed in the incubator at 37° C. and allowed to remain for 24 hours, at which time they were removed and a sample was taken from each tube with the controls examined first to validate the test. Approximately 0.05 ml of the material was removed from the tube after it had been thoroughly agitated to insure a homogeneous dispersion of the trichomonads.

The material was then placed on a 2″ x 3″ slide and covered with a 22 mm², No. 1 cover glass. The sample was observed under a microscope at low power (100X). After the number of trichomonads was observed and recorded on a protocol, the tubes were again placed in the incubator and the above procedure was repeated every 24 hours for a total period of 168 hours, at which time the test was discarded.

In all, 108 test tubes were examined for a total of 756 microscopic examinations.

Experiment 2. Effect of bacterial filtrates.

After ascertaining sterility of media and purity of strain and culture, one ml of suspended organisms was pipetted from precultures into large test tubes containing 20 ml of egg infusion broth. Four such tubes of each organism were placed on test concurrently. At the end of 24 hours incubation, one of the test tubes was removed from the incubator and growth and purity again established. The bacterial suspension was poured into a sterile Seitz filter and filtered in vacuo. The filtrate thus recovered constituted the test substance. At this time, it was decided to consider the filtrate as 100% and a dilution test was set up as shown in table V. This technique was car-

ried out on 24-, 48-, 72-, and 96-hour cultures. A test of this type was time consuming, so it was deemed conclusive to repeat the entire test only once. By using the only organism which had shown some sort of inhibition upon *T. vaginalis* when grown together as was determined in experiment 1, much time and material were saved.

After the filtrate dilution test had incubated for 24 hours, the tubes were removed from the incubator and examined microscopically for growth of the trichomonads. These data were recorded and the tubes were incubated for an additional 24 hours after which a second microscopic examination was made and the results again recorded. At the end of these two examinations (48-hour incubation) the test was deemed conclusive.

In order to give the bacteria every benefit of the situation, it was decided to pre-condition the egg-infusion medium with the bacteria, then filter the bacteria from the medium and use this filtrate as a substrate. The bacteria were inoculated into large test tubes as before and allowed to incubate for 24 hours. The bacteria were filtered out and the filtrate was dispensed into sterile small test tubes in 4-cc amounts. The trichomonads were added to the filtrate (1 ml trichomonad suspension per tube) and allowed to incubate at 37° C. for 24 hours, at which time a microscopic examination was performed and results were recorded. This reading was repeated after an additional 24-hour incubation, and so on, until 168 hours of incubation had lapsed. The results were then deemed conclusive and the test was discarded.

The above two methods of filtrate testing, dilution of the filtrate in new media and use of filtrate as a substrate, were repeated. However, instead of filtering living cultures of bacteria, the cultures to be tested were inactivated in a 60° C. water bath for one hour, at which time they were removed and filtered through a Seitz filter.

Experiment 3. Effect of *Micrococcus epidermidis* filtrate upon *T. vaginalis* in Shaffer-Frye medium.

M. epidermidis was selected for this experiment because of its effect upon *T. vaginalis* in experiments 1 and 2. One ml of the bacterial suspension from the most active of 4 pre-culture tubes (24-hour culture) was inoculated into a large test tube containing 20 ml of

egg-infusion medium. This culture, after incubation at 37° C. for 48 hours, served as a source of inoculum for 8 large test tubes, each containing 20 ml egg-infusion medium. After incubation at 37° C. for 24 hours, two of these cultures were removed: One was filtered as a living culture, while the other was inactivated in a water bath at 60° C. for one hour. By removing two cultures every 24 hours, 24-, 48-, 72-, and 96-hour living and heat-inactivated filtrates were made available as test substances.

One ml per tube of each of the eight filtrates was added to two tubes of Shaffer-Frye medium, which was prepared as for routine cultures, and then one ml of trichomonad suspension was added to each of the 16 tubes. Trichomonads were also inoculated into 2 tubes containing Shaffer-Frye medium to serve as a control. All tubes were incubated at 37° C. for 48 hours at which time they were removed and microscopic examinations were made. One examination at 48 hours was considered sufficient to test efficiency of the suspected antibiotic.

OBSERVATIONS AND RESULTS

The growth of *T. vaginalis* was surprisingly similar in all tests. In comparing the growth of controls with those tests performed to determine a growth curve, it was found that they correlated almost to the exact hour.

There is no evidence of a lag phase, which is typical of many bacteria and protozoa. The flagellate grew rapidly until it reached a level of parasitemia where it leveled off for approximately 24 hours. At 48 hours, the parasite number started diminishing until at 168 hours only a few parasites remained.

It will be seen in tables II and III that *T. vaginalis* and the bacteria, with the exception of *Organism "t"*, grew very well in egg-infusion-serum medium. The fact that *Organism "t"* grew rather slowly in egg-infusion medium is apparently peculiar to this strain because in routine cultures of *Endamoeba histolytica* with *Organism "t"* as the monoflora, turbidity is not apparent until after 72 hours incubation, and then only slight.

Experiment 1. Culture of bacteria with *T. vaginalis*

Table III shows that the bacteria in most instances showed luxuriant growth and had little or no effect upon *T. vaginalis*. However,

six of these bacteria, viz. *B. polymyxa, B. subtilis, E. coli, E. coli neopolitana, E. paragruenthali,* and *M. epidermidis,* had sufficient effect to warrant further testing. A few additional effects, not readily ascertained from the table, will be presented individually in order to describe the results more clearly.

A. aerogenes and *B. brevis* both grew well and overgrew *T. vagnialis* after 72-hour incubation. Growth of *T. vaginalis* (table III) was greatly diminished when cultivated with *B. polymyxa.* This organism produces the antibiotics Polymyxin A, B, C, and D. *B. subtilis,* which also produces a number of antibiotics (subtilin, subtilysin, eumycin, and endosubtilysin) had a similar effect upon *T. vaginalis.*

E. coli, E. coli var. *neopolitana* and *E. paragruenthali* (table III) showed apparent inhibitory effects at the end of 48-hour incubation. The effect is thought to be primarily mechanical by overgrowth. *E. coli* produces penicillinase and the antibiotic colicine. The only effect shown by *K. capsulata* was a perceptible slowing of the trichomonad motility.

M. epidermidis showed the most pronounced action against *T. vaginalis* of all the organisms tested. No flagellates were present at the end of 24 hours incubation (table III). Replicate tests gave the same results. *Ps. aeruginosa* grew very rapidly and soon overgrew *T. vaginalis.* Many reports of the antibiotic ability of this organism are to be found in the literature. It is of interest to note the inability of *S. lutea* to produce yellow pigment under the conditions of this experiment. Shaffer and Frye (16) reported that filtrates of the species of Streptobacillus used in this experiment would support the growth of *T. vaginalis* in their medium. Present results are in agreement with theirs.

It is, thus, to be seen that 6 of the 17 entities of bacteria used in this experiment were effective in inhibiting the growth of *T. vaginalis* when they were cultured with it.

Experiment 2. Effect of bacterial filtrates.

Filtrates of *Bacillus polymyxa, B. subtilis, Escherichia coli, E. coli* var. *neopolitana, E. paragruenthali,* and *Micrococcus epidermidis,* shown in experiment 1 to be inhibitory to growth of *T. vaginalis,* were used in this experiment.

It was noted that growth of the bacteria in larger test tubes was just as heavy as it was in smaller tubes. As shown in table IV, there was no appreciable difference between heat inactivated and living filtrates in their effect upon *T. vaginalis*. The dilution technique failed to reveal that any of the filtrates had an effect upon *T. vaginalis*. The known antibiotic producers were apparently ineffective against *T. vaginalis*. However, table V indicates that the filtrate of *M. epidermidis,* when used as a substrate in toto, had a suggestive inhibitory action. It will be further noted that the heat-inactivated filtrate had slightly less inhibitory action than the filtrate of the living culture. This might suggest that the active inhibitory factor is at least slightly thermolabile. Although the difference here is not too pronounced, the writer feels that it is worthy of further tests.

Table IV shows that there was no perceptible difference in filtrates with reference to their age except that the 96-hour filtrate, when used as a medium, in all cases failed to support as good growth as the control medium. The phenomenon was thought to be a consequence of the prolonged growth of the bacteria in the medium, wherein the nutrients were mostly metabolized and the medium had an accumulation of waste products.

Experiment 3. Effect of *Micrococcus epidermidis* filtrate upon *T. vaginalis* in Shaffer-Frye medium.

M. epidermidis grew very well in the egg-infusion medium and, apparently, produces a filterable substance which is inhibitory to growth of *T. vaginalis*. Dilution tests showed that this substance is present in very minute amounts (tables V and VI).

Table VI reveals that results of dilution tests in egg-infusion media are essentially the same as when the filtrate is diluted in Shaffer-Frye medium. Although the dilutions were slightly greater in Shaffer-Frye medium, results were deemed comparable to those in egg-infusion medium.

SUMMARY

1. The growth of *Trichomonas vaginalis* and 17 species of bacteria was established in an egg-infusion medium with the addition of 10% horse serum.

2. Observations were made on the effect of each of 17 bacterial organisms when grown with *T. vaginalis* in vitro.

3. It was found that six of these organisms, *B. polymyxa, B. subtilis, E. coli, E. coli* var. *neopolitana, E. paragruenthali,* and *M. epidermidis* had an inhibitory effect upon the protozoon.

4. Filtrates of the six active organisms were treated in four different ways:

(1) living cultures of bacteria were filtered
(2) heat inactivated cultures were filtered
(3) dilutions of the filtrate were tested
(4) the filtrate was used as a medium in toto.

This procedure was repeated with 24-, 48-, 72-, and 96-hour cultures of bacteria.

5. One of these organisms, *M. epidermidis,* was found to be of interest when its filtrate, used as a substrate, failed to support growth of *T. vaginalis* comparable to growth of controls.

6. Shaffer-Frye medium was used as a testing medium for filtrates of *M. epidermidis.* These filtrates were found to be ineffective at dilutions as little as 1:7.

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TABLE I

Preparation of Balamuth Egg Yolk Infusion.

1. Mix 72 grams of dehydrated egg yolk with 72 cc. of distilled water. Add 250 cc. 0.85 NaCl solution. Thoroughly mix in a Waring blender.
2. Cook in the double boiler, 20 minutes.
3. Add 40 cc. of distilled water to offset evaporation less. Cool and strain through a cheesecloth bag. Bring the volume back to 250 cc with 0.85 saline.
4. Autoclave extract 20 minutes at 15 lbs. A yellow coagulant will then be separated from the liquid. Cool below 10° C.
5. Filter through a Buchner funnel with hard filter paper (No. 50) overlaid with a G-3 filter pad. Filtrate should be clear.
6. Add to filtrate an equal amount of M/15 potassium phosphate buffer. Prepare buffer solutions as follows:

   1M dibasic potassium phosphate, K_2HPO_4
   174.180 gm/liter of dist. water
   1M potassium diacid phosphate, KH_2PO_4
   136.092 gm/liter dist. water
   These molar solutions are mixed in a ratio of 4.3 parts of K_2HPO_4 solution to 0.7 parts of KH_2PO_4 solution. To 1 part of this mixture is added 14 parts of distilled water to prepare the M/15 buffer.

7. Add 0.5% liver extract, Lilly (No. 408).
8. Tube into desired amounts.
9. Before medium is used, add 10% horse serum.

TABLE II

Bacterial Growth in Egg-Infusion-Serum Medium.

Bacteria Species	Hours Incubation at 37° C. 24	48	72	96	120	144	168
A. aerogenes	*XX	XX	XX	XX	XX	XX	XX
B. brevis	XX	XX	XX	XX	XX	XX	XX
B. circulans	XX	XX	XX	XX	XX	XX	XX
B. megatherium	XX	XX	XX	XX	XX	XX	XX
B. polymyxa	XX	XX	XX	XX	XX	XX	XX
B. subtilis	XX	XX	XX	XX	XX	XX	XX
B. subtilis (Morphotype) Panis	XX	XX	XX	XX	XX	XX	XX
E. coli	XX	XX	XX	XX	XX	XX	XX
E. coli var. neopolitana	XX	XX	XX	XX	XX	XX	XX
E. paragruenthali	XX	XX	XX	XX	XX	XX	XX
Kleb. capsulata	XX	XX	XX	XX	XX	XX	XX
M. epidermidis	XX	XX	XX	XX	XX	XX	XX
Organism "t"	0	0	X	X	X	X	X
P. vulgaris	XX	XX	XX	XX	XX	XX	XX
Ps. aeruginosa	XX	XX	XX	XX	XX	XX	XX
Sarcina lutea	X	X	XX	XX	XX	XX	XX
A streptobacillus species	XX	XX	XX	XX	XX	XX	XX

*XX—turbidity
X—slight turbidity
0—no turbidity

TABLE III

Growth of *T. vaginalis* when grown alone and with bacteria in egg-infusion-serum medium.

	GROWTH OF T. VAGINALIS Hours Incubation at 37° C. 24	48	72	96	120	144	168
A. aerogenes	XXX	XXX	XX	x	x	—	—
B. brevis	XXXX	XXXX	x	x	x	—	—
B. circulans	XXXX	XXXX	XXXX	XXX	XX	X	X
B. megatherium	XXXX	XXXX	XXXX	XX	X	X	X
B. polymyxa	X	X	x	x	—	—	—
B. subtilis	X	X	x	x	—	—	—
B. subtilis (morph.) panis	XXXX	XXXX	XX	X	x	x	x
E. coli	XX	x	—	—	—	—	—
E. coli var. neopolitana	XX	X	—	—	—	—	—
E. paragruenthali	XX	X	X	X	x	x	—
Kleb. capsulata	XXXX	XXXX	XXXX	XXX	XXX	X	X
M. epidermidis	—	—	—	—	—	—	—
Organism "t"	XXXX	XXXX	XXXX	XX	XX	x	x
P. vulgaris	XXXX	XXXX	XXXX	XX	XX	X	X
Ps. aeruginosa	XXXX	XXXX	X	x	x	x	—
Sarcina lutea	XXXX	XXXX	XX	X	x	x	x
A streptobacillus species	XXXX	XXXX	XX	X	X	X	x
Control	XXXX	XXXX	XXXX	XX	X	x	x

Trichomonads per low power field: XXXX = over 40; XXX = 21-40; XX = 11-20; X = 1-10; x = less than 1; — = none.

TABLE IV

Effect of bacterial filtrates upon *T. vaginalis* growth when diluted in egg-infusion medium.

BACTERIA	Filtrate type	Dilation	Trichomonad Growth at 24 hrs. Filtrate Age 24	48	72	96	Trichomonad Growth at 48 hrs. Filtrate Age 24	48	72	96	Control Hours 24	48
B polymyxa (1)	Living	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
	Heat Inact.	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
B. subtilis (2)	Living	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
	Heat Inact.	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
E. coli (3)	Living	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
	Heat Inact.	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
E. coli var. neopolitana (4)	Living	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
	Heat Inact.	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
E. paragruenthail (5)	Living	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
	Heat Inact.	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
M. epidermidis (6)	Living	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
	Heat Inact.	1:5	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX

Trichomonads per low power field: XXXX = over 40; XXX = 21-40; XX = 11-20; X = 1-10; x = less than 1; – = none.

TABLE V

Results of *T. vaginalis* growth when grown in bacterial filtrates.

Columns 1, 2, 3 of Table IV*	Trichomonad Growth At 24 Hrs.				Trichomonad Growth At 48 Hrs.				Trichomonad Growth At 72 Hrs.				Trichomonad Growth At 96 Hrs.				Trichomonad Growth At 120 Hrs.				Trichomonad Growth At 144 Hrs.				Trichomonad Growth At 168 Hrs.			
	Age Filtrate				Age Filtrate				Age Filtrate				Age Filtrate				Age Filtrate				Age Filtrate				Age Filtrate			
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
1.	*XXXX	XXXX	XXXX	XXXX	XXX	XXX	XXX	XX	XX	X	X	X	X	X	X	X	x	X	X	X	x	x	x	x	x	–	–	–
	XXXX	XXXX	XXXX	XXXX	XXX	XXX	XX	XX	XX	X	X	X	X	X	X	X	X	x	x	x	x	x	x	x	–	–	–	–
2.	XXX	XXX	XX	XX	XX	XX	XX	X	XX	XX	XX	XX	X	X	X	X	x	x	–	–	–	–	–	–	–	–	–	–
	XXXX	XXX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	X	X	x	X	x	x	x	x	–	–	–	–	–	–	–	–
3.	XXX	XXX	XXX	XX	XX	XX	X	X	XX	XX	X	X	X	X	X	X	x	x	x	x	x	–	–	–	–	–	–	–
	XXX	XXX	XXX	XXX	XX	XX	X	X	XX	XX	XX	XX	X	X	X	X	x	x	x	x	–	–	–	–	–	–	–	–
4.	XXXX	XXXX	XXX	XXX	XX	XX	XX	XX	XX	XX	XX	XX	X	X	X	X	x	x	X	x	x	x	x	x	–	–	–	–
	XXX	XXX	XXX	XXX	XX	XX	XX	XX	XX	XX	XX	XX	X	X	X	X	x	x	x	x	x	–	–	–	x	–	–	–
5.	XXX	XXX	XX	XXX	XX	XX	XX	X	XX	XX	XX	X	XX	XX	X	X	X	X	X	x	x	–	–	–	x	x	–	–
	XXXX	XXXX	XXX	XXX	XX	XX	X	X	XX	XX	XX	XX	XX	XX	XX	X	X	X	X	x	x	x	–	–	–	–	–	–
6.	XX	X	X	X	x	x	x	x	x	x	x	x	X	X	x	–	x	x	x	x	–	–	–	–	–	–	–	–
	XX	XX	XX	XX	X	X	X	X	X	X	X	X	X	X	X	X	X	x	X	X	x	x	–	–	–	–	–	–
CONTROL	XXXX				XXXX				XXX				XX				X				x				x			

* First three columns are same as in table IV. Symbols for amounts of growth are same as in table IV.

TABLE VI

Comparison of diluted filtrates of *Micrococcus epidermidis* when tested in Shaffer-Frye medium vs. egg-infusion-serum medium.

Filtrate type	Testing Medium	Dilution Tested	Trichomonad growth at 48 hrs. Filtrate Age 24	48	72	96	Control growth at 48 hrs.
Living	Egg-Infusion	1:5	*XXXX	XXXX	XXXX	XXXX	XXXX
Heat Inact.	Egg-Infusion	1:5	XXXX	XXXX	XXXX	XXXX	XXXX
Living	Shaffer-Frye	1:7	XXXX	XXXX	XXXX	XXXX	XXXX
Heat Inact.	Shaffer-Frye	1:7	XXXX	XXXX	XXXX	XXXX	XXXX

* Trichomonads per low power field: XXXX = over 40.

THE FLORA OF THE SHADES STATE PARK, INDIANA, AND THE GEOGRAPHICAL DISTRIBUTION OF THE SPECIES

By JACK MCCORMICK

The great number of deeds issued in west-central Indiana within a few years of the completion of the original land survey of 1820 justifies the supposition that the fertile till-soils of the region were an alluring indemnification for the hardships necessarily borne by the first colonists. The fertility of the land, however, was concomitantly to result in the decimation of the forest-covered acres of that portion of the State within half a century. Record (40) in a resume of forest conditions in Montgomery County, Indiana, related that, "Trees and saplings were cut and their trunks were made into corduroy roads. Regular logging bees were held and tree after tree was cut, rolled together, and burned. The best (trees) were cut into rails or hewn into sills, or used for firewood."

The dissected land bordering Sugar Creek and its tributaries, however, provided little enticement to the farmer; and thus their forests escaped the ruthless destruction that befell those of the surrounding uplands. One such area, which was spared denudation, became known as the "Shades of Death." Its name was an illusion, not only to the deep shadows beneath the nearly unbroken canopy of the forest, but also to the crude murders which occurred in the area within a few decades of its settlement. While the land was purchased with the more suitable uplands, much of it was left idle and virtually untouched. The title to the land passed from family to family until several holdings were consolidated and a corporation, known as the Garland Dells Mineral Springs Association, was formed to exploit the natural mineral waters (Blatchley, 2). Within a decade of the turn of the century, the resort became the property of Joseph W. Frisz. A love of the land caused Frisz, the "pioneer conservationist," to reinvest much of his profit in the land, until by 1942 "The Shades Scenic Park" comprised more than 2,100 acres (Roll, 41).

When Joseph Frisz died in 1939, his corporation stock was divided among his children. With several stockholders, each possessing a

different opinion of operational procedure, the park became a liability. When the Shades was offered for sale, "Timber companies immediately evinced great interest in the magnificent trees, especially the top-grade white oak. . . . Thus, the Shades seemed doomed for destruction," (Indiana Department of Conservation, 31). A campaign to "Save the Shades" was initiated immediately and found the sympathetic support of the school children, fraternal organizations, and other citizens of Indiana and her neighboring states. The park was thus dedicated on July 18, 1948, as the "Shades State Park," the fifteenth such area set aside in Indiana.

Various widely known botanists have visited the Shades since the late 1800's. The only known data published regarding its plant life, however, are brief notes by Deam (12) and Friesner (27). Soon after its acquisition by the Indiana Department of Conservation, it was apparent that a botanical survey was necessary to determine the types of plants present in the park, and to determine what measures might be necessary for the preservation of the less common species. This survey was initiated under the auspices of the Division of Lands, Waters and State Parks, Indiana Department of Conservation, and the Botany Department of Butler University while the author served as resident naturalist.

SIZE AND LOCATION OF THE AREA

The Shades State Park occupies an area of 1,952 acres situated in Brown Township, Montgomery County; Howard Township, Parke County; and Jackson Township, Fountain County, Indiana. (T. 17 N., R. 6 W., Sections 2, 3, 4, 5, 9, 10, 11; T. 18 N., R. 6 W., Sections 33, 34). It is approximately 5 miles north of Waveland, 50 miles west-northwest of Indianapolis, and 125 miles south-southeast of Chicago.

GEOLOGY, PHYSIOGRAPHY AND SOIL FEATURES

The Shades State Park lies within the Tipton Till Plain described by Malott (37), an area which was subjected to glaciation by both the Illinoian and Wisconsin (Tazewell substage) glacial stages. The soil of the uplands, primarily Miami silt loam, is derived from glacial and loessial materials. It is known locally as "sugar-tree land" on account of the predominance of *Acer saccharum* (Jones and

Orahood, 33). The soil of the ridges and ravines has been variously derived from the bedrock, glacial debris, and organic remains.

Bedrock consists of intermixed strata of shales, limestones, and sandstones of the Mississippian and Pennsylvanian eras. "The Mansfield sandstone (Pennsylvanian), being more resistant than the strata above and below it, has weathered into . . . bold cliffs . . ." reaching an extreme exposure of two hundred feet above deeply entrenched Sugar Creek (Jones and Orahood, 32). The tributaries of the main stream have their origin in, or are fed by, springs flowing from points where an impervious shale layer is exposed. These streams, in their adjustment to the level of Sugar Creek, have cut ravines of various width and depth. Where the springs must flow over a broad surface for any distance, beds of travertine, or tufa rock, often are formed. The extreme elevation on the park property is 771 feet above mean sea level, while the minimum elevation is slightly less than 550 feet.

FLORA OF THE PARK

During the summers of 1950 and 1951, frequent excursions were made into all sections of the Park. Prudent collections were made of each species found in order not to disturb the natural conditions. In the course of the field work, 329 new county records, including 2 species, 6 varieties, and 10 forms new to the State, were discovered. Many of these have been published, and the remainder will be published, in the Proceedings of the Indiana Academy of Science as a part of the Indiana Plant Distribution Records (23, 24).

Specimens of plants reported as State or county records were deposited in the herbarium of Butler University, while many of the other specimens are now in the herbarium of Rutgers University, the State University of New Jersey, and the private herbarium of Mr. Julius Cohen, Newark, N. J.

The nomenclature followed is that employed by Fernald in Gray's Manual of Botany, Eighth Edition (25), the most recent manual treating this region. The present study of the vascular flora of the Park comprises 627 taxonomic entities (531 species, 56 varieties, 39 forms, and 1 hybrid) including representatives of 326 genera and

98 families (table I[1]). In addition, 23 species are known to be persisting after cultivation but are not reproducing.

The major divisions of vascular plants, Pteridophyta and Spermatophyta, are represented by 30 and 597 entities, respectively. The subdivision Gymnospermae has 4 representatives, while the class Monocotyledoneae has 141, and the class Dicotyledoneae has 452 representatives. In the latter class, 54 entities are of arborescent habit and 39 are shrubs or woody climbers.

The family Compositae has the greatest number of local representatives, 86 entities. Gramineae, with 71 representatives, ranks second. Other families, in the order of the abundance of their representatives, are: Leguminosae, 33; Cyperaceae, 29; Polypodiaceae, 27; Labiatae, 17; and Scrophulariaceae, 17.

DISTRIBUTION AND STATUS IN INDIANA

The range of 613 species known to occur in the Shades was determined from the maps presented by Deam (12) and from the subsequently published records of the State Flora Committee of the Indiana Academy of Science (13-24). Forty-five per cent (276) of the species of known distribution are found to be common to all six Indiana floral areas. As a result of the location of the Park, all the species which appear in its flora are recorded as occurring in the Tipton Till Plain; 93% occur in the Illinois Drift plain, 92% in the Unglaciated area, 88% in the Lakes area, 64% in the Lower Wabash Valley, and 61% in the Prairie area.

The status of plants, whether native or introduced, was determined by reference to Deam (12) and Fernald (15). Five hundred and twenty species (83%) are considered native to Indiana; the remaining 107 species have been variously introduced.

DISTRIBUTION IN THE UNITED STATES

The national distribution of 604 species was ascertained from the accounts given in Gray's Manual of Botany, Eighth Edition (Fernald,

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[1] Table I of the original MS is on file in the Botanical Library of Butler University and is available upon loan. A mimeographed check list of the 627 taxonomic entities is available from the Butler University Botanical Library upon request.

25), the Flora of Indiana (Deam, 12), several other state and regional floras, and the periodical literature. Areal distribution was plotted and has been described by employing the cardinal points and their primary intermediates.

Sixty-eight per cent (413) of the species which occur in the Shades State Park occur in areas in each of the eight compass directions from the State and are considered, therefore, to be intraneous (Cowles, 10). One hundred and ninety-one species are extraneous, i.e., not recorded from one or more of the eight directions. Well over half of the extraneous species (118) are recorded as occurring in all but one of the eight directions.

Forty species reach their northern limit, 24 reach the southern limit, 7 reach the eastern limit, and 3 reach the western limit of their range in Indiana. Limits of distribution in the intermediate directions can be ascertained with less certainty because of the limited detail of the data available. It may be concluded, however, that the affinity of the flora of the Shades is least with the southwestern and northwestern regions.

DISCUSSIONS

The vascular flora of the Shades State Park includes a larger number and variety of species than had been anticipated at the inauguration of the study. While the survey was as intensive and extensive as the prevailing circumstances permitted, several portions of the Park remain to be investigated. There is little doubt that other species, especially members of the Cyperaceae, do occur within the boundaries of the Shades.

Extraneous species have been estimated to comprise from 40 to 45% of the total flora of Indiana (Deam, 11; Friesner, 27), 40% of the ferns and fern allies (Clevenger, 6), 62% of the grasses (Cook, 7), 56% of the shrubs (Trefz, 47), and 41% of the trees (Lindsey, 36). The extraneous element in the flora of the Shades is a consistently smaller portion of each group. The difference between the figures presented for the Park and those cited for the State ranges from 8 to 40%. Extraneous species comprise 32% of the total number of species, 23% of the ferns and fern allies, 22% of the grasses, 44% of the shrubs, and 33% of the trees found in the Shades.

These variations may be attributed to several factors: 1) Over 60% of the extraneous species in the flora of the State fail to reach the Tipton Till Plain, the region in which the Shades State Park is situated (Friesner, 27). 2) The majority of the previous estimates was made from 15 to 30 years ago. Range extensions of a number of species have been published since that time. The two most comprehensive compilations of specific ranges to appear for some time have been published within the past two years: Gray's Manual of Botany, Eighth Edition (25), and the Manual of Grasses of the United States, Second Edition (Hitchcock, 29). 3) The Shades has no natural pond or lake in which hydrophytic species, several of which are extraneous, could establish. The Park's two artificial lakes, created by raising small dams, apparently are either unfavorable habitats for those species, due, perhaps, to significant fluctuations in their water levels which may occur several times a year, or have not been in existence long enough to allow for the migration and ecesis of a sufficient number of hydrophytes to initiate hydrach succession. (4) The known flora of the Shades is composed of only about one-fourth of the total number of entities known to occur in the State (Deam, 12). As other species are discovered, the percentage of extraneous species in some groups may be altered significantly.

Nearly one-half of the species found in the Shades are present in every botanical area in Indiana. This number will no doubt be increased as our knowledge of the flora of the State becomes more complete. It is notable that the flora of the Shades has more species in common with the Illinoian Drift Area and the Unglaciated Area than the other floral regions (excepting the Tipton Till Plain). This situation might be expected, since the topography and geology of those areas resembles that of the Park to a degree. Outcrops of Mansfield sandstone occur in both the Areas. Roll (41) observed that the "deep ravines and picturesque Sugar Creek . . . (are) reminiscent of . . . the cliffs of the Muscatatuck River . . ." in the Unglaciated Area.

The fact that one-sixth of the flora of a tract set aside as "a part of original Indiana" (Cougill, 8) is made up of species which are not native to the State, and in a majority of the cases are not native to North America, bears striking evidence to the rapidity and thoroughness with which such plants are invading our land. The first intro-

duced plant probably entered the area occupied by the Park less than 120 years ago. The proportion of non-native species in the flora of the Shades is slightly greater than the 14% cited by Deam (12) for the State. The rapidity of introduction may be somewhat excelled in an area visited by thousands of persons from all parts of the United States each year. Any peculiarity due to such a condition is compensated by the distance of the Park from heavily traveled highways. The major portion of the introduced species is found in disturbed areas which have been created either by agricultural activities or by the construction of roads, clearings, or other facilities.

The new county records discovered during the course of this investigation may be grouped into three categories: those which merely "fill in" the previously known distribution of a species in the State; those which represent a minor western, southern, or northern extension of the known range of a species in central Indiana; and those which represent significant extension of the range of a species within the State. The first of these groups is by far the largest and requiries little discussion. It is exemplified by such species as *Daucus carota, Phryma leptostachya, Plantago rugelii,* and *Aster pilosus.* The second category is nearly as large as the first. The records of *Dennstaedtia punctilobula, Tsuga canadensis,* and *Bidens comosa* from Fountain County; *Pinus strobus, Atriplex patula,* and *Ranunculus sceleratus* from Park County; and *Botrychium dissectum, Oxalis europaea* var. *bushii* f. *sub-glabrata,* and *Eupatorium coelestinum* from Montgomery County are to be considered here. The third section is the smallest and perhaps the most interesting. In it may be classed the report of *Crotallaria sagittallis,* formerly recorded only from the extreme southern portion of Indiana. Here, too, may be classified the records of *Lindernia anagallidea* and *Gratiola virginica.* These species had not been reported previously from the Tipton Till Plain.

The apple of Peru, *Nicandra physalodes,* previously reported from only 10 counties, is apparently becoming a frequent weed in cornfields in and about the Park. Its close resemblance to *Datura stramonium* has caused it to be overlooked by the landowners and possibly by other botanists.

The entities added to the state floral catalog are primarily varieties and forms which have only recently come to the attention of taxo-

nomists in the State. Two species added, however, deserve particular mention:

Lespedeza cuneata (Dumont) G. Don, a native of eastern Asia, was discovered almost simultaneously in Montgomery and Parke Counties as a part of this study, and in Crawford County. It has apparently been introduced as a soil improving legume, but in neither instance had the site been seeded to the species. Its source is not known to the local residents.

Houstonia minima Beck, a species native from western Illinois to Iowa and Kansas and southward to Arkansas and Texas, grows in an undeveloped section of the Park in an intensively pastured field now dominated by poverty-grass, *Danthonia spicata*. This new eastern station may possibly herald a natural migration of the species. If this species is migrating eastward, it should have been intercepted previously in sites much nearer its native range. Apparently, no observation of this occurrence has been made. It may be more plausible to suppose, therefore, that the species has been introduced by humans or other animals. The inconspicuous habit of the plant and the situation in which it occurs, well removed from the recreational areas and dwellings, eliminates the possibility that it was formerly cultivated. The chance that it has been introduced in stock rations is very slight, since the feed has been produced on the farm for a number of years. The size of the plant is also a factor weighing against the latter possibility since most harvesting machinery would not intercept a plant so low. In addition, its fruiting period is early, not occurring at the time of harvest. It is highly improbable that wild or domesticated animals could retain the seeds in their digestive systems long enough to allow them to be transported to the farm from the area in which the species is native. Seeds may have been introduced in dung-gloves on the hoofs of newly acquired farm animals purchased in Illinois.

The compilation of a catalog of the higher plants of the Shades State Park and the attempt to relate the flora to that of the State and the Nation is not intended as an end in itself. This work, it is hoped, will serve as the foundation for a thorough study of the vegetation of the Park. As Tansley and Chipp (46) have postulated, "Floristic study must necessarily precede and condition vegetational study. . . .

The one passes naturally into the other, and we cannot possibly obtain a complete knowledge of any plant covering without using both.

SUMMARY

1. The vascular plants of the Shades State Park, an area of 1,952 acres in west-central Indiana, were the subjects of a taxonomic investigation during the summers of 1950 and 1951. The vascular flora of the park was found to consist of 531 species, 56 varieties, 39 forms, and 1 hybrid—a total of 627 taxonomic entities. In addition, 23 species have been found persisting after cultivation.

2. Forty-five per cent of the entities which occur in the Park are found common to all six Indiana floral areas. With the exception of the Tipton Till Plain, in which the Shades is located, the Illinoian Drift region has the greatest representation in the park flora. The Prairie Area has the smallest representation.

3. Seventeen per cent of the species found in the Park are considered to be alien to Indiana.

4. Extraneous species, those not found in every direction from Indiana, comprise 32% of the flora of the Shades.

5 Forty species found in the Park reach the northern limit, 24 reach the southern limit, 7 reach the eastern limit, and 3 reach the western limit of their geographical range in Indiana.

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A SURVEY OF FIFTEEN FOREST STANDS IN THE EARLY WISCONSIN DRIFT PLAIN OF INDIANA[1]

By J. Johanna Jones

The fifteen stands presented in this paper were studied to determine present-day forest composition on the Early Wisconsin Drift Plain. This geological region is an area of good farmland and consequently has relatively smaller forested area than other botanical areas of the state. For this reason it has not been given much detailed study in the past.

METHODS

The two criteria used in the selection of stands were: Lack of disturbance in recent years, and fairly even distribution over the Early Wisconsin Drift Plain. Stands A, D, J, K, M, and N are classified forests; stand O is a nature preserve. Data for stands G, H, and I were contributed by Dr. Ray C. Friesner from unpublished data. The author participated in the field work for collection of all other data.

One hundred-square-meter quadrats were laid out and all stems above 1 inch DBH. were recorded by diameter. Those below 1 inch DBH. but over knee height were recorded by number present. Twenty or twenty-five quadrats were set up per stand.

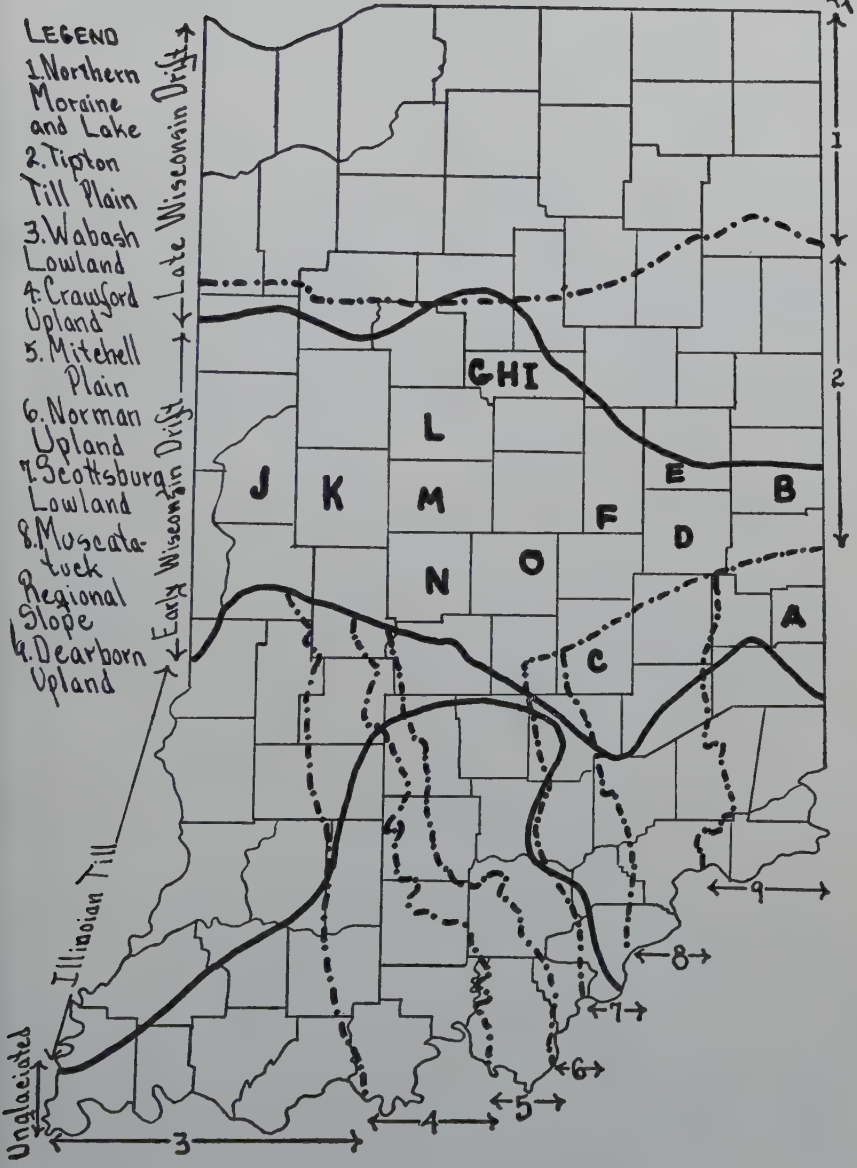
A census showing presence or absence of forb species was made in late spring and early summer and again during late summer and early autumn on all stands except G, H, and I. Presence of species was noted but no attempt was made to determine abundance or frequency index.

GEOGRAPHICAL LOCATION OF STANDS

The Early Wisconsin Drift Plain is the southernmost extension of Wisconsin glaciation in Indiana and is bounded on the north by the Union City Moraine. Leverett and Taylor (9) publishing in 1915,

[1] A portion of a thesis submitted in partial fulfillment of the requirements for the degree Bachelor of Arts, Magna cum Laude, in Butler University.

FIGURE 1 PHYSIOGRAPHIC MAP OF INDIANA
after Malott: Handbook of Indiana Geology



state that the division between Early and Late Wisconsin is based on topographical differences thought to show age differences. They discard the terms due to insufficient evidence. Malott (11) publishing in 1922, recognized the division because north of the Union City Moraine the ice sheet broke into independently moving lobes while to the south it had advanced and retreated as a whole. More recent references to the division were not found.

Boundaries of the Early Wisconsin Drift Plain (after Handbook of Indiana Geology) and locations of stands on it are indicated in figure 1. This area includes most of the Tipton Till Plain and parts of the Dearborn Upland, Muscatatuck Regional Slope and Scottsburg Lowlands, each of which comprises a different physiographic area. Those touched upon in this paper are characterized as follows: Tipton Till Plain, flatness; Muscatatuck Regional Slope, dissected but less so in north due to overlay of Wisconsin glacial till; Dearborn Upland, dissected, probably less so to the north. Even though Wisconsin glacial till modifies to some extent in their northern parts the characteristics of the areas bordering the Tipton Till Plain, these areas present a habitat different from the Tipton Till Plain.

All but two stands studied are located within the Tipton Till Plain and all are located within the Early Wisconsin Drift Plain. Stand A (Union, Union Co.) is within the Dearborn Upland, and stand C (Ray's Crossing, Shelby Co.) is within the Muscatatuck Regional Slope, in the Tipton Till Plain.

Key to names and locations of stands is as follows:

A Union, Union County (DU)
B Lynn, Randolph County (TTP)
C Ray's Crossing, Shelby County (MRS)
D Dunreith, Henry County (TTP)
E Hancock, Delaware County (TTP)
F Goss, Madison County (TTP)
G Ross, Howard County (TTP)
H Robard, No. 1, Howard County (TTP)
I Robard No. 2, Howard County (TTP)
J Cade, Fountain County (TTP)
K Oliver, Montgomery County (TTP)
L Smith, Clinton County (TTP)
M Randel, Boone County (TTP)
N Davis, Hendricks County (TTP)
O Buzzard's Roost, Marion County (TTP)

RESULTS

Woody Species

Results for woody species are shown in table I and figure 2. Table 1 lists for each species of each stand the numbers of specimens by size class, frequency index, basal area, percentage of stems 9 inches DBH. and over of each species in relation to all species, and total number of stems over 1 inch DBH.

On the basis of woody species, stands fall into the following classes: Moist soil, characterized by Ulmus and Aesculus; Transition, characterized by Ulmus and Fagus; Mesophytic, characterized by Acer and Fagus; and Dry, characterized by Quercus, Carya and Fagus. Stands are so grouped in figure 2. Principal dominants are grouped by general habitat preference with most mesophytic species at top and moist soil species at bottom. Bars represent the percentage which the number of stems 9 inches DBH. and over of each species bears in relation to total number of stems 9 inches DBH. and over for all species in each stand. The fifteen species considered most indicative of forest type and having representation in the 9-inch-DBH.-and-over class are shown in figure 2. This percentage is used to indicate relative importance of species with large enough stem diameter to compete effectively in crown control. Basal area, while figured for all species tabulated, was not used for this purpose for the following reasons: 1. It would not indicate correct relationship between stands since on a few stands twenty, not twenty-five, quadrats were run. 2. It would not indicate relative numbers of trees; a given number of square inches might be one or a few very large trees or a large number of small ones.

Fifty-eight species of trees and twelve of shrubs and lianas are recorded. In moist soil stands *Ulmus americana* is abundant; Aesculus, Platanus and *Acer saccharum* are present. In transition stands Fagus is abundant and Ulmus is still well represented. This is in accordance with Potzger (13) who states that Fagus enters lowlands as soon as soil is a few inches above the water table. In mesophytic stands *Acer saccharum* and Fagus are abundant. In dry stands Quercus and Carya are abundant, and a surprising amount of Fagus is present.

FIG.2

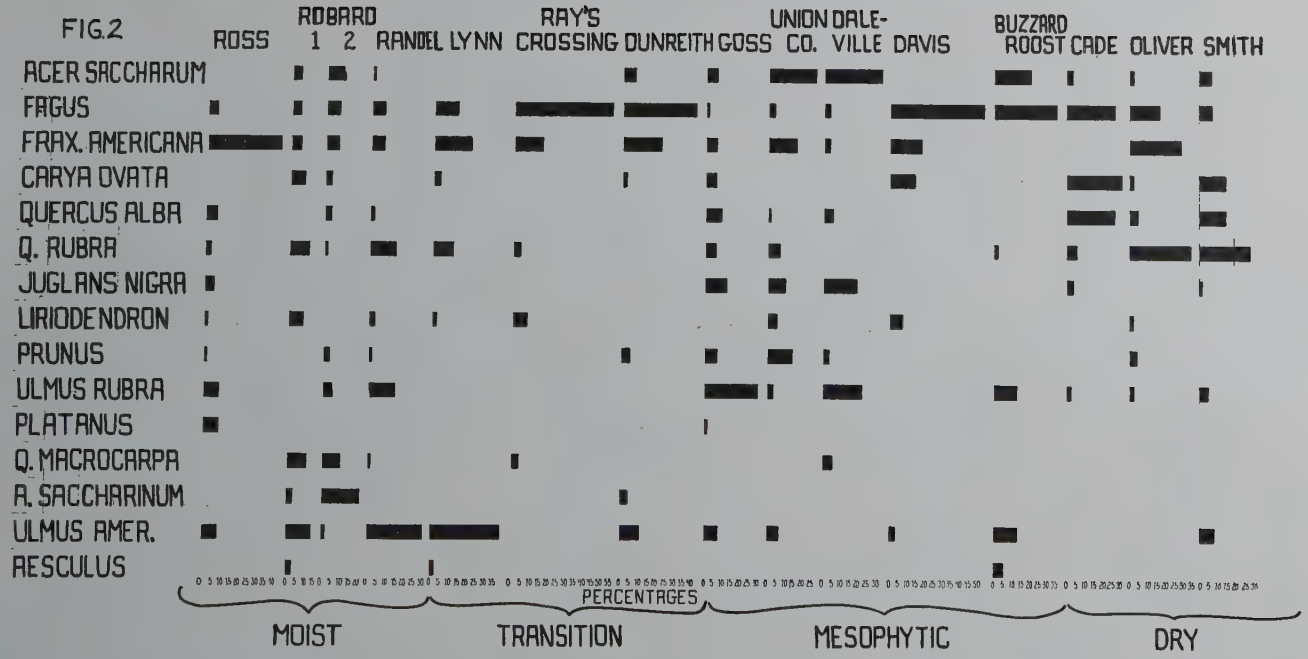

ROSS
ROBARD 1
ROBARD 2
RANDEL
LYNN
RAY'S CROSSING
DUNREITH
GOSS
UNION CO.
DALE-VILLE
DAVIS
BUZZARD ROOST
CADE
OLIVER
SMITH
ACER SACCHARUM
FAGUS
FRAX. AMERICANA
CARYA OVATA
QUERCUS ALBA
Q. RUBRA
JUGLANS NIGRA
LIRIODENDRON
PRUNUS
ULMUS RUBRA
PLATANUS
Q. MACROCARPA
A. SACCHARINUM
ULMUS AMER.
AESCULUS
PERCENTAGES
MOIST
TRANSITION
MESOPHYTIC
DRY

Figure 2 shows that a high percentage of *Fraxinus americana* is associated in general with high percentage of *Fagus grandifolia* and/or *Acer saccharum* from which one might assume that the first-named species preferred mesophytic environment. Stand G (Ross, Howard Co.) therefore poses a problem with high percentage of Platanus, Ulmus and *Fraxinus americana*. Differentiation, however, was not made between *F. americana* and *F. pennsylvanica v. subintegerrima* (green ash) since the distinguishing characteristic present in winter is the bud, and this is out of reach. Therefore any reference to *F. americana* might include specimens of the green ash which appears to prefer more moist soil. In stand G this is probably the case.

Stand I (Robard's No. 2, Howard Co.) is interesting because of the high percentages of both *Acer saccharum* and *A. saccharinum*, indicators of well drained and poorly drained soils respectively. The author was not present when data for these stands were gathered, but study of data sheets indicates that all specimens of *A. saccharum* were in a small area; the frequency index of *A. saccharum* was 20% and that of *A. saccharinum* 48%. Presence of *A. saccharum* was therefore probably due to edaphic factors of limited extent, possibly a rise in ground producing better drainage. The significance of this and other types of micro-climate are discussed by Potzger (14).

Stand O (Buzzard's Roost, Marion Co.) shows higher percentages of *Ulmus americana* and *Aesculus glabra* than would be expected with its high percentages of *Acer saccharum* and Fagus. Topography, however, is hilly, and the stand is adjacent to Fall Creek. Moist-soil species are found in the deeper valleys.

Stands J, K, and L (Cade, Fountain Co.; Oliver, Montgomery Co.; and Smith, Clinton Co.) form a very interesting group, in which *Acer saccharum* and Fagus decline in abundance and *Carya ovata, Quercus alba,* and *Q. rubra* assume *greater importance*. Such a stand of oak-hickory may be due to prairie influence which invades in this area according to Deam (3). Although top-soil of the three dry-species stands was loamy, a cut-away hillside in the Cade woods exposed underlying sand. Potzger (15) in his study of the early surveyor's records in Indiana shows a striking abundance of Oak-Hickory in this same area with corresponding decline of Beech-Maple. Gordon (8) has included this area in the Upland Oak Forest in his vegetation map on Indiana. Stands J, K, and L are not typically

Oak-Hickory; they have abundant representation of such mesophytic species as Fagus, *Acer saccharum* and *Fraxinus americana*. Braun (1, p. 186) notes a tension zone between the Eastern part of the Oak-Hickory region and the western part of Beech-Maple. Such a tension seems well-expressed in these stands particularly in stands J and K where dissected areas permit both dry and mesophytic habitats. It will be noted that abundance of beech and white ash is much lower in the flat stand L. This tension zone extends disjunctly into central Indiana where it finds expression because of microclimate induced by exposure of slope (Potzger, 12) and on into Ohio where in certain places the original forest was oak according to Miss Braun.

The prairie influence is shown by Potzger and Keller (18) in their study of the beech line in northwestern Indiana. Abundance of beech is shown by township for twenty-two counties. Counties which lie on the periphery of the peninsula include Fountain, Montgomery, and Clinton. In peripheral counties Fagus is represented by 30 to 50% of forest cover on the side distal to prairie and less than 5% or none on the prairie side. Stands J, K, and L are found in this most dynamic region of the above-mentioned tension-zone.

As previously noted, stand C (Ray's Crossing, Shelby Co.) is within, and stand D (Dunreith, Henry Co.) in on the edge of, the Muscatatuck Regional Slope. Both these stands are conspicuous for their high percent of Fagus. This is in striking accord with the findings of McCoy (10) for the northeast Illinoian Till Plain. The stands studied by McCoy, however, lay well within the Illinoian Till Plain and characteristically contained good representation of *Liquidambar styraciflua* not found in the stands of the Early Wisconsin Drift Plain. That habitats of the northern, Wisconsin-glaciated part of the Muscatatuck Regional Slope may be subject to much the same conditions as southern Illinoian-glaciated part is indicated by Malott (11) who states that the northern surface of the Slope is composed of Wisconsin Drift which is quite thin. Stands C and D fall into the area classified by Gordon (8) as Beech Forest in his vegetation map of Indiana.

Of all areas studied, stand M (Randel, Boone Co.) was perhaps the least mature, and J, K, and O (Cade, Fountain Co.; Oliver, Montgomery Co.; and Buzzard's Roost, Marion Co.) the most nearly mature. Upon consideration it was felt that both extremes should be

included to form an accurate picture of current vegetation. Table 1 clearly indicates merging between moist and mesophytic species of the region. Few of the woods studied were of a dry type nor would many be expected in this flat area which has at least in spots a close underlayer of Illinoian Till Plain clay. On the other hand, better drained land has been generally utilized for agricultural purposes, leaving timber only around water-logged areas. This paper reiterates what Friesner (6) has shown, that Indiana is a critical botanical area of varied plant species.

Forb Species

Forbs occurring in all stands are shown in table II.[2] A total of 196 species were recorded. Of this total only three species, viz. *Geum canadense, Podophyllum peltatum* and *Viola papilionacea* occurred in all stands. Eight additional species, viz. *Calium circaezens* v. *hypomalicum, G. concinnum, Geum canadense, Sanguinaria canadensis, Sanicula canadensis, Tovara virginica, Urtica dioica* v. *procera,* and *Viola pensylvanica* v. *leiocarpa* were recorded from eleven of the twelve stands whose forbs were recorded.

The number of forb species present in each stand is as follows:

A	Union, Union County	68
B	Lynn, Randolph County	69
C	Ray's Crossing, Shelby County	52
D	Dunreith, Henry County	37
E	Hancock, Delaware County	40
F	Goss, Madison County	45
J	Cade, Fountain County	84
K	Oliver, Montgomery County	76
L	Smith, Clinton County	59
M	Randel, Boone County	63
N	Davis, Hendricks County	70
O	Buzzard's Roost, Marion County	68

Order of increasing number of forb species is D, E. F, C, L, M, A and O, B, N, K, and J. Stands of J and K, both classified as dry, have highest number of forb species. No other direct correlation between number of forb species and forest type occurs.

[2] Table II of the original thesis, not printed in this paper. The original thesis is available on loan from the Butler University Botanical Library.

Forbs found only in stands classified as moist are:

1. Iris virginica v. shrevei
2. Lysimachia nummularia
3. Potentilla simplex
4. Ruellia strepens

Species 1 and 2 are indicated as moist soil types by Fernald (5).

Forbs found only in dry stands were:

1. Anemone virginiana
2. Desmodium glutinosum
3. D. nudiflorum
4. Solidago ulmifolia
5. Chelone glabra v. linifolia
6. Dioscorea villosa
7. Monarda clinopodia
8. Rumex verticillata
9. Sium suave
10. Bidens vulgatus
11. Lactuca canadensis v. longifolia
12. Lobelia inflata
13. Mitella diphylla
14. Senecio obovatus

Species 1 through 4 are noted by Fernald as dry soil types. Species 5 through 9, however, are noted as moist-soil species. These latter species may be explained by micro-climate induced by ravines in stands J and K and by a depression in woods L.

The following were noted particularly: *Agrimonia gryposepala* in stand K is a southern outpost of the present Indiana distribution. *Dioscorea quaternata* has a southern distribution in Indiana; presence in stands A, and B represent its most north-ward Indiana extension. *Lilium canadense* shows scattered southern distribution; Union County is on the northern boundary of its range. *Monarda clinopodia* is on the northern limits of its range in Fountain County where it is found in stand J.

DISCUSSION

Woody Species

The forest climax of an area is the expression of the climax modified by edaphic and physiographic factors. Climate is relatively constant in the area here considered, so that edaphic and physiographic factors are the variants. The climate places Indiana within the range of a number of species which demand different soil conditions. Various combinations of these species are therefore brought about by different edaphic conditions. An association is, after all, a dynamic and ever-varying combination of plant species, never static but continually changed by the elimination and entrance of different species. It is even possible that individuals of the same species may possess genes rendering them fit for different habitats. Thus we find ash,

elm, and silver maple important in the moist areas; beech the first of the climax representatives to attain high abundance in the forests transitional between moist and mesophytic; sugar maple and beech attaining high abundance in the mesophytic stands; and sugar maple and beech both descreasing in abundance as oaks and hickories increase in dry, western stands. In spite of this merging tendency, crown cover exclusive of stands J, K, and L (Cade, Fountain Co.; Oliver, Montgomery Co.; and Smith, Clinton Co.) displays strong similarity; all stands contain beech and most of them contain sugar maple and ash. Influence of valleys bringing in lowland species such as Aesculus and Ulmus is apparent in mesophytic and dry stands. To the west the prairie influence is striking as indicated in stands J, K, and L (Fountain, Montgomery and Clinton Counties). According to Braun (1) all stands of this study except J and possibly K would be included in the Beech-Maple region, which has as its southern boundary the southern boundary of Wisconsin glaciation. South of the Beech-Maple region she places the Mixed Mesophytic, characterized by large numbers of dominants: beech, tuliptree, basswood (*Tilia heterophylla*), sugar maple, chestnut, sweet buckeye, red oak, white oak, hemlock, and others. All these and others are found in the present study with the exception of *Tilia heterophylla, Aesculus octandra*, chestnut and hemlock. The first two are replaced by *T. americana* and *A. glabra*, while the chestnut has been eradicated by blight. Potzger and Friesner (17) have called Mixed Mesophytic the ultimate climax of Indiana and placed Berkey Woods, in northern Indiana near Warsaw, into this classification. This is certainly a northern extension of the Mixed Mesophytic area of Braun.

In interpreting the data of this study the age of the area must be kept in mind. The Wisconsin Drift Plain is young (due to recent glaciation) having been exposed to plant invasion for much shorter time than the Illinoian glaciated area to the south and perhaps millions of years less than the unglaciated areas. The Mixed Mesophytic forest of Braun is the result of these millions of years of plant habitation. In Michigan the Beech-Maple forest attains high purity; for example a stand in Emmett County, Michigan, with as high as 98.3% comprised of beech and maple together and 1.7% by Tsuga. The data for this stand are published by Braun (1, p. 352) and labelled typical Beech-Maple. Cain (2) presents data for a Michigan stand with 81.5% abundance of beech and maple. With these extremes in mind

we must consider the situation of the Early Wisconsin Drift Plain. At the time of glaciation all plants were wiped out and new inhabitants entered by migration and remained by ecesis. Migration was from the unglaciated area so that new invaders appeared first in the south, moving gradually north. Otto (12) shows how Acer and Fagus followed after colder climate trees, such as Abies and Picea, into the Drift Plain. As a result a dynamic transition zone between Beech-Maple and Mixed Mesophytic has been set up. This area tends toward an ultimate Mixed Mesophytic climax except perhaps for prairie areas to the west. Stands of the Early Wisconsin Drift Plain lying between the extremes of unglaciated region Mixed Mesophytic and Michigan's Beech-Maple show high number of species though perhaps not so high as often found farther south and show great importance of beech and maple as illustrated in the Berkey Woods mentioned above.

There usually is a transition zone at the junction of any two associations. The junction of Beech-Maple and Mixed Mesophytic is further complicated due to successive glaciations, so that forests of the junction zone are in different stages of development and the soils of different compositions. One cannot expect to draw a boundary between the two associations but must rather recognize a gradual transition from one to the other, ever influenced by microclimate and species ranges. The author classifies the stands of Early Wisconsin Drift Plain of Indiana as transitional between Mixed Mesophytic and Beech-Maple; they may be called mixed hardwood.

Stands considered in this paper are of secondary succession; virgin forests were not available. Potzger (15) however, shows the same general results in his maps compiled from the early surveyors' records of the forest primeval. Abundance of oak and hickory was low in the Early Wisconsin Drift Plain except for counties bordering the prairie peninsula where it increased markedly. Abundance of Acer, Fagus, and Fraxinus was high throughout the Plain until the prairie peninsula was approached, when these species dropped out.

Forb Species

Aster and Desmodium in particular were found in dry stands more often than in moist. The families Leguminosae and Compositae are typical indicators of prairie influence. It is interesting that the woods studied by Esten (4) though located in Parke County and

therefore in the same region as stands J, K, and L of this paper, lacks Solidago and Aster, and has very slight representation of the legumes. This emphasizes the difference in forbs of a Beech-Maple stand such as that studied by Esten and the drier stands J, K, and L (Fountain, Montgomery and Clinton Counties).

SUMMARY AND CONCLUSIONS

1. Quadrat data for fifteen stands within the Early Wisconsin Drift Plain are presented.

2. Results of a forb census taken in late spring and early summer and again in late summer and autumn are presented. Dry stands J and K are found to have highest number of forb species.

3. Stands fall into the categories: Moist soil, characterized by Ulmus and Aesculus; Transition, characterized by Ulmus and Fagus; Mesophytic, characterized by *Acer saccharum* and Fagus; and Dry, characterized by Quercus, Carya, and Fagus.

4. All stands except A (Union, Union Co.) and C (Ray's Crossing, Shelby Co.) are located within the Tipton Till Plain; A is within the Dearborn Upland and C within the Muscatatuck Regional Slope. Stand D (Dunreith, Henry Co.) is just outside the Muscatatuck Regional Slope in the Tipton Till Plain.

5. The Early Wisconsin Drift Plain of Indiana is felt to be within a transition zone between Beech-Maple to the north and Mixed Mesophytic to the south.

6. An outstanding Oak-Hickory group on the western part of the area studied may be due to proximity of prairie area and to sandy sublayer.

7. Forest stands of this paper, although of secondary succession, are very like the forest primeval as represented by Dr. J. E. Potzger in his study of early surveyors' records.

8. Beech is the most important dominant, with fair to abundant representation in all stands. Sugar maple and ash are next in importance.

9. The Early Wisconsin Drift Plain is primarily a physiographic zone rather than vegetational although the two concepts are interde-

pendent. Immediate modification of vegetation is seen in stand C which is within the Muscatatuck Regional Slope rather than the Tipton Till Plain.

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TABLE I—WOODY COMPOSITION

Stand A: Union, Union Co.; B: Lynn, Randolph Co.; C: Ray's Crossing, Shelby Co.; D: Dunreith, Henry Co.; E: Hancock, Delaware Co.; F: Goss, Madison Co.; G: Ross, Howard Co.; H: Robard No. 1, Howard Co.; I: Robard No. 2, Howard Co.; J: Cade, Fountain Co.; K: Oliver, Montgomery Co.; L: Smith, Clinton Co.; M: Randel, Boone Co.; N: Davis, Hendricks Co.; O: Buzzard's Roost, Marion Co.

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
Acer negundo	A	1								4		
	B	1	1	1					2	12	7.0	
	C	4	1		1				2	20	41.6	
	D	3								5		
	H				1				1	4	78.5	2.7
A. rubrum	A				1				1	4	28.2	
	B	4	6	6	3				15	32	179.8	
	K				1				1	4	50.2	
	M							1	1	4	855.3	1.7
A. saccharinum	D	25	12	3	2	1		2	20	40	1080.2	4.5
	G	6	2	1					3	4	23.5	
	H							1	1	4	1413.7	2.7
	I	1	1	1	3	2	4	6	17	48	4744.6	21
	L	12		1					1	4	19.6	
A. saccharum	A	348	62	46	29	3	1		141	100	2888.6	27
	B	44	27	5	2				34	72	245.0	
	C	264	169	70	10				249	100	1392.5	
	D	37	1	3	1		1	2	8	65	1111.8	6.7
	E	394	372	46	6	3	4		431	100	2937.7	33
	F	427	19	9	4	2			34	90	539.5	6.1
	G	431	25	7	1				33	76	413.1	
	H	3	2					2	4	24	1767.9	5.4
	I		1		1	4	1		7	20	982.5	10
	J	323	104	18	4				126	100	845.8	3.8
	K	335	177	78	14	1			270	100	1797.0	2.1
	L	137	6	1	1	1	1	1	11	64	896.9	6.9
	M	313	137	5	3		1		146	92	542.7	1.7
	N	364	110	7	2				119	100	98.1	
	O	1723	180	46	11	4	3	2	246	100	3418.6	21

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
Aesculus glabra	A	2								8		
	B	5	5			1			6	20	118.5	1.9
	C	11	7						7	28	7.8	
	D			1	1				2	5	35.3	
	E	9	11	1					12	32	22.7	
	F	26	20	8					28	45	89.5	
	G	17	3		1				4	24	57.3	
	H						1		1	4	314.1	2.7
	K	8								24		
	M	28	11	2					13	32	28.2	
	O	69	2				2		4	64	794.0	5.7
Asimina triloba	B	1								4		
	C	149	6						6	60	7.0	
	D	1								5		
	G	41	1	1					2	20	13.3	
	H	84	4						4	12	5.4	
	I	66	6	4					10	28	44.7	
	J	59	5						5	36	5.4	
	L	77	13						13	8	10.2	
	M	129								40		
	N	84	5						5	50	3.9	
	O	8								12		
Carpinus caroliniana	A	21	3	1	1				5	48	66.7	
	B	49	39	4	1				44	88	110.7	
	C	7	18	9					27	56	129.0	
	D	6	3	2					5	20	30.6	
	E		1						1	4	.7	
	F	21	6						6	20	5.4	
	G	38	8	5					13	52	52.0	
	I	3	1						1	12	12.5	
	J		5	1					6	16	18.0	
	K	3	6						6	8	4.7	
	L	37	2	1					3	36	18.8	
	M	37	26	7					33	56	81.6	
	N	63	11	2	1				14	55	73.0	
	O	1								4		
Carya cordiformis	A	4	2	4	2				8	28	150.3	
	B	8	6	1					7	32	23.5	
	C	4						1	1	20	415.4	4.0
	D				2				2	10	78.5	2.2
	E				1	1			2	8	226.9	2.8
	F	6	1	1		1	1	1	5	35	691.9	4.5
	G	3	2	1					3	24	16.4	
	H	6				2	1		3	32	494.0	5.4
	I				1	1			2	8	226.9	1.7
	J	3	1						1	16	.7	
	K	12	1	3	1				5	32	38.4	
	L	4								8		
	M	3	1	2					3	20	25.9	
	N	16	1	4					5	60	31.4	
	O	9	1						1	28	.7	
C. glabra	J		1						1	4	.7	
C. laciniosa	C							1	1	4	380.1	4.0
	F	2				1			1	15	132.7	1.5

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
C. laciniosa	H					2			2	8	289.8	5.4
	I				1	1			2	8	191.6	3.4
	M	7	8	6	4		1		19	52	454.3	1.7
C. ovalis	J		1						1	4	.7	
	K	3								8		
C. ovata	B	6	4	16	9		1		36	68	776.7	3.9
	C		2	1					3	4	10.9	
	D					2			2	10	208.1	2.2
	E					1			1	4		
	F			1		4			5	20	537.2	6.1
	G	5	4	5	4				13	28	203.4	
	H	9				2	1		3	16	427.4	8.1
	I			1	4				5	16	227.7	3.4
	J	4	23	10	16	7	1		57	84	1979.6	31
	K	22	3	4	3				10	52	115.3	2.1
	L	13		1	6	2	1	5	15	56	1067.4	15
	M	2								8		
	N	16	25	35	11	4			75	60	1175.7	14
	O				1				1	4	28.2	
Celastrus scandens	L	1								4		
	O	1								4		
Celtis occidentalis	A	14			1				1	16	63.6	1.6
	C	1			1				1	8	258.3	4
	E	19	8	5	3				16	64	181.4	
	F	18	6	1					7	30	18.8	
	G	10								8		
	H	4		1					1	12	20.4	
	J	2								8		
	M	10	1						1	24	10.2	
	N	2								10		
	O	5	1	4	1	1			7	40	287.4	3.8
Cercis canadensis	A	12								4		
	E			1					1	4	12.5	
	F		1						1	5	.7	
	J	2	1	2					5	8	17.2	
	K		5						5	4	6.2	
Cornus alternifolia	A	3								4		
	G		1						1	4	.7	
C. drummondi	A	2								8		
	B	23								16		
	F	4	4						4	15	3.1	
	G	180	14						14	48	35.3	
	H		1						1	4	.7	
	I	10	1						1	12	3.1	
	N	9								10		
C. florida	A	21	2	1					3	40	23.5	
	B	3								4		
	C	3		1					1	12	7.0	
	D	7	1	4					5	28	44.2	
	E	3	1						1	12	.7	
	G		2						2	4	1.5	
	J		1	1					2	4	10.2	
	K	20	3	1					4	36	11.5	
	L	24	7						7	52	5.4	

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
C. florida	M			2					2	8	19.6	
	N	9	1						1	20	.7	
Corylus americana	M	10								4		
Crataegus sp.	A	1								4		
	B	5								16		
	C	2		1					1	12	7.0	
	D	2	6						6	15	4.7	
	F	3	2						2	10	1.5	
	G		1	1					2	8	20.4	
	H	20	5	2	1				8	56	63.6	
	I	16	8	3					11	28	41.9	
	J	2								4		
	K	2								4		
	L	1	2						2	8	1.5	
	M	13	1	2					3	32	20.4	
Euonymus atropurpureus	A	1								4		
	C	1								4		
	D	2								5		
	E	3								8		
	F	5								10		
	G	5	1						1	16	.7	
	H	1								4		
	L	2	1						1	4	.7	
	M	2								8		
	N	6								20		
Fagus grandifolia	A	8	5	11	2	2			20	48	596.9	4.8
	B	11	16	21	9	3	2		51	68	2754.8	13
	C	4	20	6	2	2	8	4	42	76	4020.4	56
	D	4	6	1	2	2	4	13	28	65	12330.5	42
	E	1	1					1	2	8	380.9	2.8
	F	1	1		1				2	15	81.6	1.5
	G	2	3				2	1	6	20	1020.2	5.0
	H		2					2	4	12	802.6	5.4
	I	21	2				1	3	6	20	1476.5	6.8
	J	1	2	1	3	4	8	3	21	40	3886.2	27
	K	13	2	5	5	5	2		19	76	1551.9	17
	L	23	2			1	1	3	7	36	1568.4	6.9
	M	12	2		1			3	6	32	1432.5	6.8
	N	41	19	2	2	3	3	8	37	75	16599.0	54
	O	2	2		1	1	8	10	22	56	6343.6	36
Fraxinus americana	A	48	2	4	8	2	3		19	56	1665.3	16
	B	63	8	21	16	9	1		55	96	3748.3	21
	C	33	11	11	5	3	1	1	32	68	1706.8	16
	D	36	1		4	8	1		14	75	1434.9	22
	E	2		1	1	1			3	20	175.9	2.8
	F	31	7	7	4	1			19	80	522.2	6.1
	G	46	3	17	34	11			65	84	3151.3	42
	H	10	5	1	1	1	1		9	48	464.9	5.4
	I	2	6		1	2	1		10	36	650.4	6.8
	J	23		1					1	40	7.0	
	K	23	5	22	40	3			70	88	2580.3	29
	L	137	56	8	2				66	96	333.3	
	M	188	11	7	7	3			28	96	652.1	6.8

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
Fraxinus americana	N	138	14	3	3	3	1		24	100	908.7	18
	O	21	4	1	1				6	32	62.8	
F. americana v. biltmoreana	I							1	1	4	706.8	1.7
F. nigra	D					1			1	5	132.7	2.2
	I		1		3	2			6	16	413.8	5.1
F. quadrangulata	B			1					1	4	12.5	
	E	5	3						3	28	4.7	
	F	6			1				1	25	38.4	
	G				2				2	4	142.1	3.3
	I					1			1	4	259.1	3.4
	K		1	2					3	8	14.8	
	N	16	2						2	45	1.5	
	O	6	4						4	24	10.2	
Gleditsia triacanthos	E				1				1	4	63.6	2.8
	F		1						1	5	.7	
Gymnocladus dioica	L	2	1	1	1				3	8	80.8	1.7
Juglans cinerea	F			1	1				2	5	69.9	
	K	1								4		
	N				1				1	5	78.5	3.6
	O	1								4		
J. nigra	A			2	3	2	1	1	9	20	1144.3	9.5
	B				1				1	4	50.2	
	C	1	2						2	12	3.9	
	D				1				1	5	50.2	
	E				1	4	1	1	7	16	1218.1	19
	F	2		2	4	4	2		12	40	1299.8	12
	G			1	1	2	1		5	20	590.6	5.0
	I	1								4		
	J				1		1		2	8	279.6	3.8
	L							1	1	4	380.1	1.7
	M	1								4		
	N			1	2				3	5	76.1	
Juniperus virginiana	A	1								4		
Lindera benzoin	A	5								4		
	B	22								20		
	C	40								32		
	D	246								85		
	F	67								40		
	G	307								84		
	H	386	10						10	84	7.8	
	I	57								48		
	K	2								4		
	L	429	3						3	92	2.3	
	M	353	3						3	76	2.3	
	N	98								55		
	O	1								4		
Liriodendron tulipifera	A	2		3	3	2	1	1	10	40	1017.2	4.8
	B	1				1			1	8	95.0	1.9
	C	1		1		2			3	16	284.3	8.0
	G					1			1	4	132.7	1.7
	H	2		1			1	2	4	16	1312.4	8.1
	K						1		1	4	201.0	2.1
	L		1						1	4	.7	

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
Liriodendron tulipifera	M							2	2	8	660.5	3.4
	N	1	4	1		2			7	25	261.5	7.1
Maclura pomifera	D	1								5		
Morus rubra	A	5	1						1	12	.7	
	B		1						1	4	.7	
	C	9	1						1	24	.7	
	E	2								8		
	F	5	4						4	20	5.4	
	G		2	1					3	8	23.5	
	J	6	2	2					4	28	10.2	
	K	18	13						13	32	8.6	
	L	3	3						3	20	2.3	
	M	3	1						1	12	3.1	
	N	2	3						3	15	2.3	
Nyssa sylvatica	A	1		4					4	4	12.5	
	B				1				1	4	28.2	
Ostrya virginiana	A	58	7	6	1				14	40	115.4	
	B	77	53	3	3				59	84	119.4	
	C	4	7	7					14	40	95.0	
	D	161	3	6		1			10	35	179.3	2.2
	E	5	24	2					26	48	150.7	
	F	152	6	2					8	65	18.8	
	G	8	8	12		1			21	40	208.4	1.7
	H	5	8	6					14	32	58.1	
	I	20	2		2				4	32	90.3	
	J	35	85	17	3				105	84	417.8	
	K	1592	9		2				11	100	94.2	
	L	42	21	1					22	68	50.2	
	M	41	21	3					24	48	58.1	
	N	383	53	10	1				64	95	201.8	
	O	25		3	2				5	40	113.0	
Parthenocissus quinquefolia	B	1								4		
	C	1								4		
	J	4								12		
Platanus occidentalis	F				1				1	5	78.5	1.5
	G				2	4	1		7	16	837.2	8.3
Populus deltoides	F					1			1	5	132.7	1.5
P. grandidentata	G							1	1	4	572.5	1.7
Prunus serotina	A	9	2	9	9	2			31	40	1003.7	14
	C	3	8	4					12	36	54.1	
	D	35				13		1	14	40	479.0	4.5
	E				1			1	2	8	529.3	2.8
	F	25	12	1	4		1		18	70	446.3	6.1
	G	21	2		1	1			4	16	80.1	1.7
	H		5	8	1				14	32	134.3	
	I	5		1			2		3	12	488.5	3.4
	J	12	6						6	24	7.0	
	K	9	5	2	2	2			11	44	404.4	4.2
	L	68	15	1					16	60	33.7	
	M	12	11	3	5		1		20	60	166.5	1.7
	N	60	3	1		1			5	50	119.3	
	O	5								8		
Pyrus coronaria	B			1					1	4	12.5	

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
Pyrus coronaria	D	1								5		
Quercus alba	A					1			1	4	113.0	1.6
	B	3	1						1	16	3.1	
	E	1						2	2	8	805.0	5.5
	F	2		2	1	4	2		9	40	1145.1	9.1
	G	5	1	2	2	2			7	20	335.3	6.1
	H							1	1	4	706.8	1.6
	I					1	1		2	8	380.9	3.4
	J	1	2	3	6	5	3	2	21	48	2815.6	27
	K	16	4	9	2			1	16	56	549.1	4.2
	L	11		1	1	1	2	5	10	44	4368.3	15
	M	3	2					1	3	20	710.7	1.7
	N	1	1						1	10	.7	
Q. bicolor	B		1	1					2	4	7.8	
	F			1	1				2	5	47.9	
	H				1		1		2	8	333.0	5.4
	I					2	1	2	5	20	1071.2	8.5
	J		2						2	8	2219.5	
	L							3	3	8	2217.9	5.2
Q. macrocarpa	C	40						1	1	8	660.5	4.0
	E						1		1	4	283.5	5.5
	H							4	4	12	2744.1	11
	I						2	4	6	20	2911.4	10
	L	2								8		
	M						1		1	4	226.9	1.7
Q. muehlenbergii	C	24								4		
	G	2			1				1	8	38.4	
	L						1		1	4	201.0	1.7
	N	14	2						2	40	1.5	
	O						1	1	2	8	717.8	3.8
Q. palustris	H							1	1	4	706.8	2.7
	M	1		1	1	3			5	20	356.5	5.1
Q. rubra	A			1	1	2	1		5	16	479.8	6.3
	B	2	2	2	2	5	2		13	44	1269.9	11
	C	3		1				1	2	16	1597.5	4.0
	E	1								4		
	F	1			2	2			4	25	355.0	6.1
	G	6		2	2	1			5	16	310.2	3.3
	H							4	4	16	2304.3	11
	I						1		1	4	314.1	1.7
	J		1			1		1	3	12	1017.0	5.8
	K	7	1	6	6	14	1		28	64	2162.1	35
	L	2				3	6	8	17	48	5034.8	29
	M		4	1		2	1	6	14	44	3001.0	15
	O	1					1		1	8	226.9	1.9
Q. shumardii v. schneckii	I						1		1	4	254.4	1.7
Q. velutina	K						1		1	4	226.9	2.1
	M					2			2	8	245.8	3.4
Rhus glabra	C	5								8		
R. radicans	B	20								36		
	H	12								24		
	I	12								28		
	J	9								16		

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
R. radicans	K	1								4		
	L	2								4		
	M	7								16		
Ribes cynosbati	A	49								36		
	B	4								4		
	C	27								12		
	D	17								30		
	E	1								4		
	G	7								20		
	H	23								20		
	I	11								8		
	J	9								12		
	K	37								36		
	L	277								84		
	M	57								44		
	N	11								15		
	O	5								4		
Rosa sp.	D	3								10		
	G	1								4		
	H	11								12		
	I	52								40		
	L	8								20		
	M	7								4		
	N	1								5		
Rubus allegheniensis	B	48								12		
	D	25								25		
R. occidentalis	A	2								4		
	B	7								8		
	C	2								8		
	D	10								25		
	F	4								5		
	G	9								4		
	N	19								5		
Sambucus canadensis	A	5								16		
	B	10								12		
	D	27								55		
	E	1								4		
	F	1								5		
	G	1								4		
	I	1								4		
	L	1								4		
Sassafrass albidum	J	1	3	2					5	16	21.9	
	K	11	5	1	5				11	32	211.2	
	L	2								8		
Smilax rotundifolia	A	5								4		
	C	18								16		
	K	1								4		
	N	15								5		
	O	2								4		
Smilax tamnoides v. hispida	A	12								24		
	B	2								8		
	C	2								4		
	D	1								5		

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
Smilax tamnoides v. hispida	E	1								4		
	F	1								5		
	G	9								20		
	H	4								4		
	J	33								12		
	K	1								4		
	L	18								24		
	M	1								4		
	N	11								20		
	O	10								4		
Staphylea trifolia	K	6	1						1	4	.7	
Tilia americana	A	4			1	2			3	12	228.5	4.8
	B	13	9	2	5	2		2	20	48	1603.0	7.7
	C	3	7	8					15	16	90.6	
	G	6		3	4	1		1	9	44	831.8	3.3
	H			3				2	5	8	1198.5	5.4
	K	7	5						5	24	6.2	
	L	72	12	4			1		17	68	275.1	1.7
	M	79	46	9	4	1		1	61	76	758.9	3.4
	N	2								10		
	O	3	1	3			1		5	8	291.3	1.9
Ulmus americana	A	11				4			4	36	398.1	6.3
	B		1	8	13	6	6		34	64	2823.4	39
	C	3		1					1	8	7.0	
	D	18			4	2			6	40	538.7	11
	E	3	3	10	3				16	36	238.5	
	F			4	5	1			10	25	476.9	7.6
	G	10	17	42	13	2	1	1	76	44	1829.7	8.3
	H	17	77	72	11	1		2	163	92	3382.5	14
	I	163	84	21				1	106	88	2349.1	1.7
	J	3	11	5	1				17	56	368.3	
	K	4			2				2	20	164.1	
	L	141	57	12	4	2	1	1	77	88	1449.0	8.6
	M	15	22	21	25	11	2	1	82	92	3765.9	31
	N	1	3	11	11				25	75	604.7	3.6
	O	25	2	2	6	2	1	1	14	64	697.4	13
U. rubra	A	57	1				2		3	44	487.7	3.2
	B	2								4		
	D	86								25		
	E	9	9	21	3	2	4	1	40	52	2120.5	22
	F	63	84	11	9	13			117	95	2960.0	30
	G	56	6	3	4	4			17	56	801.8	8.3
	H	6	7	8	5				20	44	267.8	
	I	7	1	4			2		7	40	597.6	5.1
	J	20	9	5	4	1		1	20	60	330.1	1.9
	K	23	3			1			4	28	80.8	2.1
	L	30	2			1	2		5	44	874.9	5.2
	M	25	3	5	6	6	3		23	60	2951.1	15
	N	35	1		1				2	45	29.0	
	O	6		3	6	3		1	13	36	1455.3	13
U. thomasi	A	2								4		
Viburnum acerifolium	B	11								4		
	K	6								12		

TABLE I—(Continued)

Woody Species	Stand	Below 1"	1-2	3-5	6-10	11-15	16-20	Above 20"	Total Above 1"	FI	Basal Area sq. in.	% of all above 9"
Viburnum acerifolium	N	1								5		
V. prunifolium	B	11								20		
	C	3								4		
	F	9	2						2	10	1.5	
	H	2								4		
	I	4								8		
	L	17	1						1	18	.7	
	M	45	14						14	36	10.9	
Vitis sp.	A	5								12		
	B	1								4		
	C	10								20		
	D			1					1	5	12.5	
	E	2								8		
	F	1								5		
	G	4	7						7	24	14.9	
	H	18								20		
	J	9								16		
	K	10								36		
	L	1								4		
	M	2								8		
	N	3								10		
Xanthoxylum americanum	L	69	2						2	36	1.5	

A FIVE-ACRE FOREST SURVEY AT SHADES STATE PARK (INDIANA). A STUDY OF SAMPLING METHODS[1]

By CHARLES L. TROTTER

Various methods have been used to represent the vegetation of a given area on the printed page. In dealing with an area as vast as a forest, only a representative sample of the entire vegetation can be studied conveniently. Therefore sampling methods used must be extensive enough to include at least all the important species in the woods that would be included if the entire forest could be tabulated. A sampling method should show with reasonable accuracy a representation of the number of species present, abundance, stem sizes, and the regularity of their distribution.

It is the purpose of this paper to give what is believed to be the first percentage composition data of the Shades State Park forest, and to study and compare the results obtained by using different sampling patterns in the five acre survey.

LOCATION AND TOPOGRAPHIC FEATURES OF SHADES STATE PARK

The entrance to Shades State Park lies about five miles north of Waveland, Indiana, on State Road 234. The park covers an area of 1,952 acres in Montgomery, Fountain, and Parke counties. Most of the area is in the southwest corner of Montgomery county; the tract under study lies entirely within Sec. 11, T. 17 N., R. 6 W. of Montgomery county.[2]

Montgomery county, and consequently Shades State Park, occurs in the Tipton Till Plain. Dryer (4) has described this same large area as "The Central Till Plain." According to Malott (6) "The Tipton Till Plain is characteristically a slightly modified ground moraine

[1] A portion of a thesis submitted in partial fulfillment of the requirements for the Bachelor of Arts degree, Magna cum Laude, Butler University.

[2] 39° 56′ 06″ N. Lat. crosses 87° 03′ 49″ W. Long. at the southern edge of the area sampled.

plain, and over wide areas is monotonously flat." Digression from this monotony occurs at Shades State Park. "In Fountain, Montgomery, and Parke counties considerable relief exists where the main streams have dissected the plain," (6). Between the streams, however, the till plain is "well preserved and is fairly representative," (6). "Sugar Creek in southwestern Montgomery and northern Parke counties is deeply entrenched in and below the massive resistant Mansfield sandstone, and sheer cliffs of 100 ft. or more are present," (6). "In the 'Shades of Death' (2) park and the Turkey Run State Park, Sugar Creek and its tributaries exhibit wild and rugged scenery," (6). A maximum entrenchment of over 200 ft. occurs in those areas. Complete topographic maps of the Alamo Quadrangle of Indiana (containing Shades) may be purchased for 10c by writing U. S. Geological Survey, Department of Interior, Washington 25, D. C.

According to the Purdue University Agricultural Experimental Station, Special Circular for January 1944, (3) the dominant soil types in the area studied include "Fincastle, Russel, and Cope Silt Loams and Brookston Clay Loam." Medium to heavy leaching has occurred; the subsoil is moderately permeable on sloping land and slowly permeable in flat depressions. At Shades, the deep leaf litter characteristic of a deciduous forest is present, but moderate gully erosion occurs on a few slopes.

METHODS

Weeks before the tabulation work was carried out, the author and others went into the area to be studied and laid out 144 10-meter quadrats. Stakes were driven at the corners of the quads and white string was stretched around appropriate stakes. The quadrats were then numbered at their southwest corners until, at the time of tabulation, 144 quadrats were delimited and systematically numbered on the forest floor. Quadrat number 61 was omitted from the tabulations because 143 10-meter x 10-meter areas total five acres.

All stems 1 inch DBH. or larger were measured with wooden calipers. Stems below 1 inch DBH. but at least 3 ft. in height were tabulated. Figure 1 shows six types of 10% sampling patterns (A-F), one 28% sampling pattern (G), and one 34.6% sampling pattern (H). The latter pattern consisted of 50 quadrats distributed evenly over the five acre tract.

SIGNIFICANT DATA

The total results are presented in tables I-IX,[3] which, because of their bulk, are somewhat unwieldy. Table I shows abundance, size classes, and per cent F. I. for the total number of species in the five acre stand; tables II-IX,[3] present the same sociological features for the various patterns. From these we shall select data which have the most significant bearing on the problem of accuracy of sampling methods.

A summary of variations in per cent F.I. of trees 2 inches DBH. or larger is presented in table X.[3] This table does not include sampling pattern H, the purpose of which is to show how closely the results of a 34 per cent pattern consisting of uniformly distributed quadrats corresponds with the results of the entire five acres with regard to total vegetation and crown cover. These comparisons may be made by perusal of figure 2 and tables I and IX.[3]

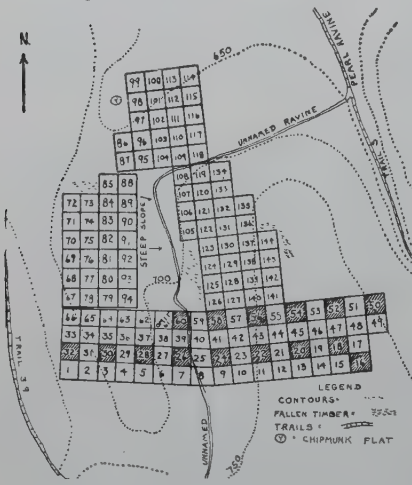
Table X highlights two important facts. First: species which show frequency indices between 10.4 (*Ulmus americana*) and 52.0 (*Fraxinus americana*) in the results of the five acre plot (table I), are absent in varying numbers in all the 10% sampling patterns, but all appear in the 28% sampling pattern (figure I-G). Secondly: F.I. varies with different sampling patterns for most species, especially for *Fagus grandifolia*. Variation is least in the 28% sampling pattern.

The forest is of the beech-mixed hardwoods type which Potzger (7) and Potzger and Friesner (8) have considered climax for Indiana. It is present on mesophytic habitats all over the state. While Fagus has 50% of the large size stems, 13 other species participate in the crown cover. Here, as in nearly all forest stands comparable to the type at Shades Park, *Acer saccharum* plays only a somewhat secondary role in the crown cover but reproduces prodigiously (table I). This stand is also typical for the Indiana climax forest in the absence of a well expressed shrub layer.

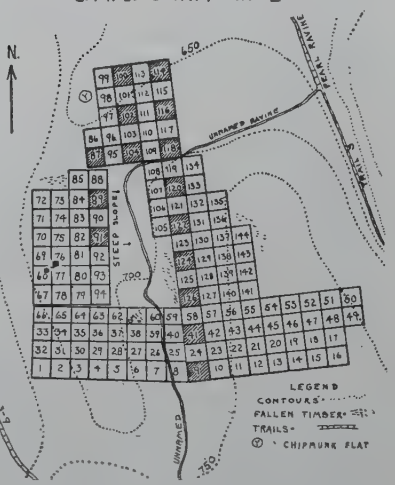
At Shades Park, the occurrence of ten species in five acres whose F.I. do not exceed 10% (table I) is attributed to what has been

[3] These are table numbers of the original manuscript which is available on loan from the Butler University Botanical Library. Only tables I and X are presented in this paper.

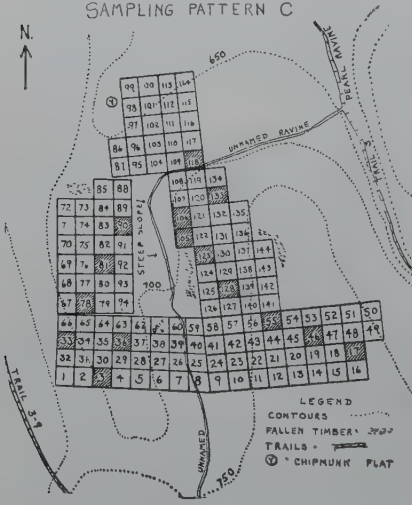
SHADED AREAS COMPRISE
SAMPLING PATTERN A



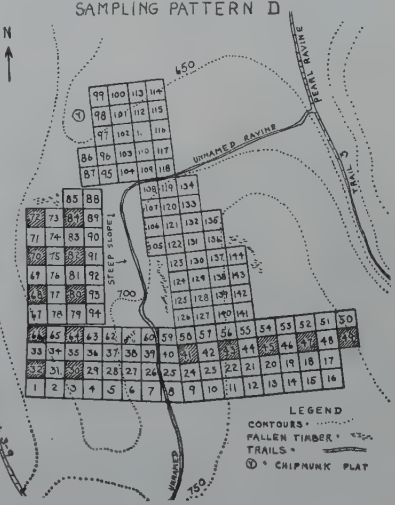
SHADED AREAS COMPRISE
SAMPLING PATTERN B



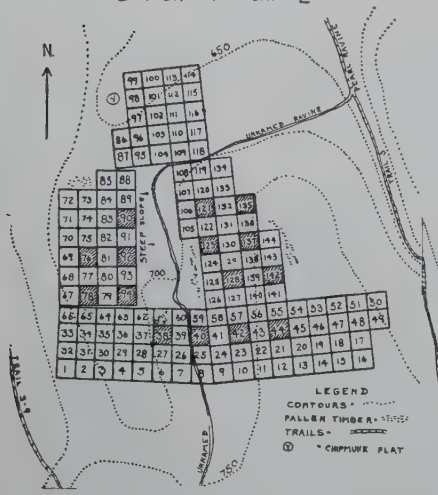
SHADED AREAS COMPRISE
SAMPLING PATTERN C



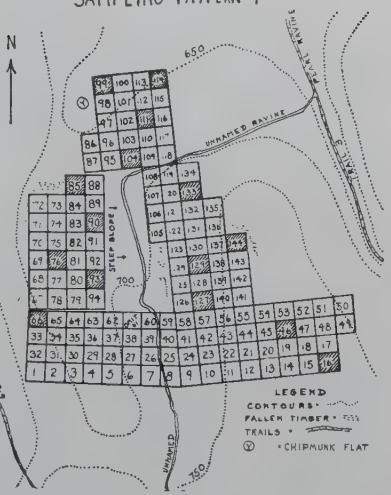
SHADED AREAS COMPRISE
SAMPLING PATTERN D



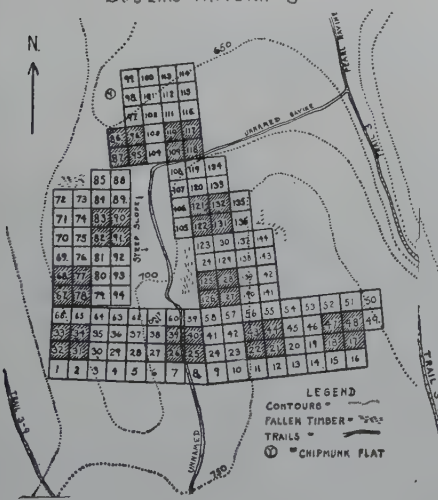
SHADED AREAS COMPRISE
SAMPLING PATTERN E



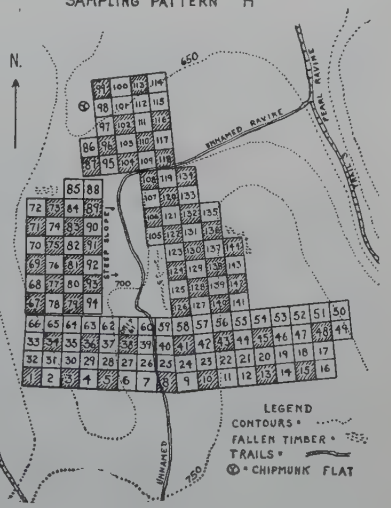
SHADED AREAS COMPRISE
SAMPLING PATTERN F



SHADED AREAS COMPRISE
SAMPLING PATTERN G



SHADED AREAS COMPRISE
SAMPLING PATTERN H



aptly termed a "mosaic of habitat," resulting in a mosaic of microclimate. While the topography of the area is somewhat rugged, some spots are very moist and subject to inundation in spring. Here Platanus and Ulmus, definitely out of place in a mesophytic habitat, find expression. On dry places on the slopes, oaks and hickories ecize and join beech and tulip poplar in the crown cover. For indication of crown control, abundance of stems 10 inches DBH. or larger was used (figure 2) because it was assumed that stems of this diameter had successfully overcome competition for light and now participated in the crown cover.

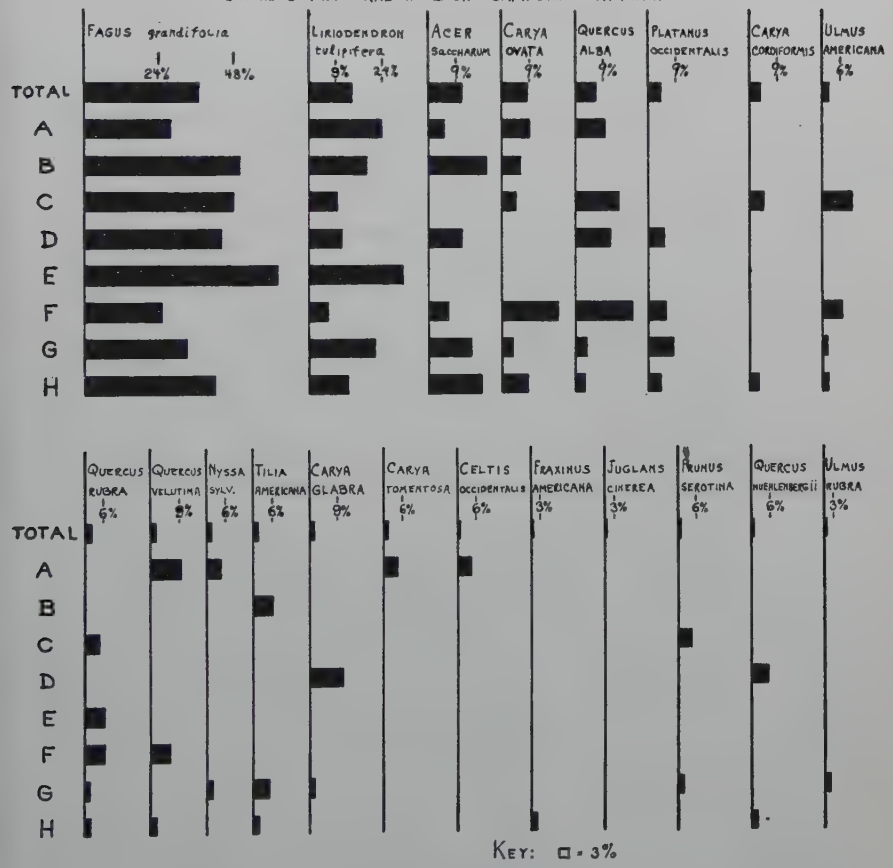
Pattern C (figure 1-C) covers all the types of habitat in the five acre stand, starting on the hill to the east and moving northwest across the ravine, southwest to the top of the hill at quadrat 33 and then back across the valley. As a result of microclimatic variations, the vegetation in pattern C shows species from the somewhat hydrophytic Ulmus (10%) to the xerophytic species *Carya cordiformis* (4.8%) and *Carya ovata* (4.9%) as indicated in figure 2. Sample E avoids the moist land by the valley and stays fairly well to the hilltops and slopes (figure 1-E). Only three species participate in the crown cover of this sample: Fagus (62%), Liriodendron (31%), and *Quercus rubra* (7%), (figure 2). Thus, whether the "mosaic of habitat" is used or carefully avoided, the type of vegetation reflects topographic selection.

DISCUSSION

Foremost among the distinguishing criteria of a mature forest is the comparatively great number of large stems. Only after decades of undisturbed growth and reproduction does a wood become mature to the extent that, although prodigious reproduction occurs in some species, only that species which is most able to adapt itself to prevailing conditions, i.e., to ecize, will survive. This results in a sort of natural selection which is controlled primarily by the macroclimate, but is varied within certain macroclimatically controlled areas by the microclimate of topographic differences which Potzger (7) has shown to be present in the rugged parts of Indiana. In a study of forest types in the Versailles State Park area, Potzger (7) shows that "Indiana is a very sensitively balanced climatic region where comparatively small differences in soil moisture induce striking differ-

FIGURE 2

PERCENT OF TOTAL STEMS 10" OR LARGER DBH IN ENTIRE 5 ACRES AND IN EACH SAMPLING PATTERN



ences in forest cover types." Results of the present study (figure 2) lend some credence to that statement.

Auten (1) considers slightly more than 200 stems per acre 2 inches DBH. or larger as indicative of a mature stand. At Shades State Park, in the present 5-acre study, there were 200 stems 2 inches DBH. or larger per acre, a fact which provides one of the best evidences that the stand is not only mature but that it has not experienced disturbances by man. As a forest matures, Griffin (5) has shown that "competition initiates elimination of numbers of individuals until an equilibrium is established between the carrying capacity of a given habitat and the number of stems in such an area."

That the 10% sampling patterns used are not always uniform in indicating crown cover is shown somewhat by the foregoing but more in detail by careful perusal of figure 2 correlated with topographic conditions for each sampling pattern (figure 1, A-F). While no attempt will be made to account for every variation in the results of the different sampling patterns, we should note that some species which have frequency indices between 10.4 and 52.0% in the five acre stand (table I) are eliminated in varying numbers in the six 10% sampling patterns. This, is, of course, what one would expect of a species which has a low F.I. in a given stand. Such species play only a minor role in the total crown cover (figure 2).

Validity of 10% sampling patterns when one is not dealing exclusively with crown cover is not altogether a different story. When based on trees 2 inches or larger DBH. (table X), species which reproduce excessively in spots, but which obviously have high mortality rates, i.e. *Fraxinus americana* and *Nyssa sylvatica*, are eliminated. The results of sampling patterns C and D more closely approach the true 5-acre tabulations than do the other 10% patterns (table X).

In the present study it is clear that a widely scattered selection of quadrats for a 10% sampling pattern does not necessarily result in a more accurate representation than a pattern arranged in a line. In fact, in the present study the pattern comprised of widely scattered quadrats (figure 1-F) results in the least accurate representation of the five acre stand of any 10% pattern used. From figure 2-F, it is evident that *Carya ovata*, *Carya glabra*, and *Quercus alba* all have

frequency indices more than twice the value they attain in the five acre stand (table I), while *Fagus grandifolia, Liriodendron tulipifera,* and *Acer saccharum* show much less abuandance than they actually show in the five-acre tabulation. Nevertheless, it is apparent (table X) that as a whole, where varying topographical features are included, the 10% sampling patterns except F give a fairly good representation of the important associates in the 5-acre stand. It is quite possible that less variation in results obtained by different 10% sampling patterns would be experienced in stands where topography is less variable. No doubt a uniform topography would automatically eliminate many of the unimportant species showing low frequency indices.

Variation in topographic complexity should no doubt be compensated by increased coverage by sampling units in this study. There is a definite increase in accuracy of representation (figure 2, G and H) when quadrats are enlarged and more area is included in a sampling pattern or when 34.6% of the 5-acres is tabulated by means of evenly distributed 10-meter quadrats. Sampling pattern G (figure 2,), which covers 28% of the 5 acres and consists of ten 20-meter x 20-meter quadrats, does not eliminate rather important species, such as *Acer saccharum, Quercus alba* and *Carya ovata* from the crown cover. It results in a representation more closely approaching the total five acre tablations of the species with 9% or greater representation than any of the 10% sampling patterns (figure 2). The results (table IX and figure 2-H) of sampling pattern H (figure 1-H) more closely approach the total 5-acre tabulations than any other pattern used. Frequency indices of species listed in table IX and those of the same species in table I are strikingly comparable. Although sampling pattern H fails to show *Nyssa sylvatica, Carya glabra, C. tomentosa, Juglans cinerea,* and *Celtis occidentalis* in the crown cover (figure 2-H), it gives an accurate picture of the tree crown cover, as comparison with figure 2—Total will quickly indicate.

It is apparent then, that in the present study, 28% or 34% sampling patterns, even though they more closely approximate results of the total tabulation, do not include all species; a few unimportant ones are eliminated, and their elimination by these more inclusive patterns attests to their unimportance. Obviously the best distribution of quadrats over the area is that whose results most clearly ap-

proach the percentages shown in the area as a whole, i. e. when the entire stand is tabulated—in this case, distributions H (34%) and G (28%).

SUMMARY

1. A five-acre forest stand at Shades State Park was divided into 143 10-meter x 10-meter quadrats, and tabulations of all woody species occuring in the 5 acres were recorded.

2. Eight sampling patterns were derived to test their accuracy in recording the known 5-acre results. Six 10% patterns (figure 1, A-F), one 28% pattern (figure 1-G) and one 34% pattern (figure I-H) were used.

3. All 10% sampling patterns, except E, and perhaps C, present a fairly accurate picture of the forest cover at Shades State Park.

4. Species showing frequency indices between 10.4% and 52% in the 5-acre tabulation (table I) were eliminated in varying numbers in the results of the 10% sampling patterns when results were based on stems 2 inches DBH. or larger (table X).

5. The 10% sampling pattern comprised of widely scattered quadrats (figure 1-F) resulted in less accurate representation than the more regular 10% patterns.

6. The results (figure 2, G and H, and tables VIII and IX) of the 28% and 34% sampling patterns (figures 1, G and H) show a closer correlation with tabulations of the 5-acre stand than any of the 10% patterns used.

7. It is suggested that in a forest stand of less topographical variation, the results of any 10% sampling pattern would perhaps closely correlate with the total tabulation.

ACKNOWLEDGMENTS

The author is indebted to Dr. J. E. Potzger for suggestion of the problem, supervision in laying out the quadrats and for critical reading of the thesis, and to Dr. R. C. Friesner for supervision of the tabulation work and critical reading of the thesis. The author is grateful to his colleagues, Johanna Jones, William Harris and Robert Petty, for help with the field work.

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TABLE I

Tabulation of Woody Species on Five Acres at Shades State Park: Diameter Classes in Inches DBH.

Trees	Diameter Classes Inches DBH. Below 1	1-2	3-5	6-9	10-15	16-20	Above 20	Total	F.I.
Acer saccharum	2,101	1,013	198	23	7	5	9	3,355	100.0
A. rubrum	14							14	4.8
Asimina triloba	229	2						231	17.5
Carpinus caroliniana	28	38	13	1				80	24.4
Carya cordiformis	8	1	4	8	4	1	1	27	13.0
C. glabra	4	2	1	1	3			11	4.8
C. ovata	11	4	6	6	11	3	1	42	18.0
C. tomentosa				1	1	1		3	1.3
Celtis occidentalis	9	2			1			12	4.8
Cornus florida	106	40	42					188	39.0
Fagus grandifolia	23	58	43	11	16	17	37	205	70.0
Fraxinus americana	151	38	3			1		193	52.0
Juglans cinerea							1	1	
Liriodendron tulipifera	5	1	3	4	14	12	1	40	15.0
Morus rubra	11	1						12	7.0
Nyssa sylvatica	505	20	1	3	2		1	532	19.5
Ostrya virginiana	105	26	5	3				139	34.0
Platanus occidentalis					2	5	1	8	4.2
Prunus serotina	11	1			1			13	4.2
Quercus alba	9	5	3	1	7	3	2	30	13.0
Q. muehlenbergii	1	1	1				1	4	2.8
Q. rubra	29	1	1	11	4			46	14.0
Q. velutina				1			3	4	2.0
Sassafras variifolium	12	3	7	3				25	11.8
Tilia americana	115	5	4	4	2	1		131	16.7
Ulmus americana	3	4	2	4	4			17	10.4
U. rubra	31	1	4	2			1	39	18.8
Total								5,402	
Shrubs									
Celastrus scandens	8							8	2.1
Cornus alternfiolia	27	2						29	6.2
Dirca palustris	7							7	2.8
Hamamelis virginiana	26							26	8.4
Lindera benzoin	209	5						214	18.1
Parthenocissus quinquefolia	1							1	
Ribes cynosbati	1							1	
Sambucus canadensis	6							6	1.3
Smilax tamm. v. hispida	21							21	10.4
Viburnum acerifolium	45							45	9.0
Vitis sp.	1	1	1					3	1.3
Total								361	
Total Trees Plus Shrubs								5,763	

TABLE X*

Comparison of F. I. of Tree Species
Two Inches DBH. or Larger According to 8 Patterns of Sampling
In a Five Acre Stand at Shades State Park

Species: Trees only	Entire Five Acres	Sampling Patterns "A"	"B"	"C"	"D"	"E"	"F"	"G"
Acer saccharum	90.0	86.7	80.0	100.0	93.3	93.3	80.0	92.5
Carpinus caroliniana	14.6		26.3	20.0	13.0	13.0	13.0	12.5
Carya cordiformis	9.8			6.6		6.6	6.6	2.5
C. ovata	15.3	20.0	6.6	6.6	6.6	13.0	26.3	17.5
Cornus florida	22.2	6.6	33.0	6.6	26.3	13.0	40.0	25.0
Fagus grandifolia	64.3	60.0	53.3	66.6	53.3	93.3	53.3	57.5
Fraxinus americana	6.3		6.6		13.0	6.6	13.0	2.5
Liriodendron tulipifera	14.6	20.0	20.0	13.0	33.3	13.0	6.6	20.0
Nyssa sylvatica	4.9	6.6		6.6	6.6			2.5
Ostrya virginiana	8.3	6.6		6.6	13.0	6.6	20.0	7.5
Quercus alba	10.4	20.0	6.6	13.0	13.0		20.0	10.0
Q. rubra	9.0	13.0		6.6	13.0	13.0	13.0	12.5
Sassafras variifolium	7.0	20.0		13.0	6.6		20.0	2.5
Tilia americana	5.6		13.0	6.6				12.5
Ulmus americana	8.3		6.6	13.0	6.6		13.0	10.0
U. rubra	5.6		6.6		6.6	6.6		7.5

* Shows only those trees which had a F. I. or 10 or over in table I.

THE PHYTOPLANKTON OF THE J. W. FRISZ MEMORIAL LAGOON, SHADES STATE PARK, INDIANA

By William A. Daily and Jack McCormick*

Plankton net collections were made from the J. W. Frisz Memorial Lagoon in the Shades State Park, Montgomery County, at various intervals of time from June 12, 1951 until April 19, 1952. Most of the collections were made by Jack McCormick, several by Jay D. Gilliland of Waveland, Indiana, and a few by W. A. Daily.

The thirty-seven year old artificial lagoon has an area of approximately three acres and a maximum depth of eighteen feet. It has been well stocked with game fish, but higher aquatic plants are scarce.

From a total of sixty collections, thirteen genera and thirteen species representing five classes of the algae have been found.

When a range of time is given with a species, it was collected daily to at least once a week during that time. Less frequent occurrence is indicated by the date of collection.

MYXOPHYCEAE

Diplocystis aeruginosa (Kütz.) Trevis.
July 11 to July 30, Aug. 16 to Sept. 1, Sept. 9, Sept. 29, and Oct. 6.

Anabaena circinalis (Kütz.) Rabenh. ex Born. & Flah.
July 1 to July 30, Aug. 16 to Aug. 23.

Aphanizomenon flos-aquae (L.) Ralfs ex Born. & Flah.
Sept. 1, Sept. 29, Oct. 6 to Oct. 20.

CHLOROPHYCEAE

Pandorina morum Bory
June 25 to July 30, Aug. 18 to Aug. 24, Sept. 29.

Sphaerocystis Schröeteri Chodat
April 19, 1952.

BACILLARIOPHYCEAE

Melosira granulata var. *angustissima* Müller

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* Present address: Department of Botany, Rutgers University, New Brunswick, New Jersey.

Identified by Dr. Ruth Patrick.

July 14 to July 30, Aug. 16 to Sept. 1, Sept. 9, Sept. 29, Oct. 6 to Oct. 20.

Synedra ulna var. *Chaseana* Thomas

Identified by Dr. Ruth Patrick.

July 1; July 3; July 14; Aug. 16 to Sept. 1; Sept. 9; Sept. 29; Oct. 6 to Oct. 20; Nov. 17; and Apr. 19, 1952.

DINOPHYCEAE

Gonyaulax palustre (Lemm.) Schiller

Identified tentatively by Dr. James B. Lackey.

June 12 to July 30; Aug. 16 to Sept. 1; Sept. 9; Sept. 29; Oct. 6 to Oct. 20; Nov. 5; Nov. 17; Apr. 19, 1952.

Glenodinium quadridens (Stein) Schiller

July 11, July 21 to July 24, Aug. 16 to Aug. 25, Sept. 29, Oct. 6 to Oct. 20.

Gymnodinium neglectum (Schilling) Lindem.

Identified tentatively by Dr. James B. Lackey.

July 21.

Ceratium Hirundinella (O. F. Müller) Schrank.

June 12 to July 30; Oct. 6 to Oct. 20; Nov. 5, Nov. 17; and Apr. 19, 1952.

CHRYSOPHYCEAE

Mallomonas alpina Pascher and Ruttner

April 19, 1952.

Dinobryon divergens Imhof.

June 14 to June 21; Dec. 8; and Apr. 19, 1952.

All of the collections are to be found on file in the Butler University Herbarium and the Cryptogamic Herbarium of the Chicago Natural History Museum.

We wish to acknowledge the aid and suggestions from Mrs. Fay K. Daily and Dr. Francis Drouet.

The Herbarium
Department of Botany
Butler University

A SYNOPSIS OF THE COCCOID MYXOPHYCEAE

By Francis Drouet and William A. Daily

During the past ten years we have examined large numbers of specimens of this group in the field and in American and European herbaria. Most of the more than one thousand type specimens involved in the nomenclature were found and studied. Many of these proved to be representatives of the twenty-eight species listed here; the remainder are material of Chlorophyceae, Rhodophyceae, bacteria, fungi, and other plants and animals. The following synopsis and keys are offered as a summary of the chief morphological features of the various taxa. It is hoped that a detailed revision, complete with synonymy and lists of specimens, can be published in a short time.

These plants are difficult of classification because, with no differentiation into hard parts, the cells vary considerably according to the nature of the environment. The species seem to be of world-wide distribution. Some inhabit only fresh water or salt water, while others grow well in both. Some are strictly planktonic, while others survive equally in shallow water, in the plankton, and in aerial habitats.

CHROOCOCCACEAE Näg.

Gatt. einz. Alg., p. 44. 1848.—Plants uni- to multicellular, of diverse shapes and sizes, free-floating or on various substrata; cells spherical, discoid, ovoid, cylindrical, or pyriform, each dividing into two cells of equal size and soon becoming separated from each other by sheaths of gelatinous material; reproduction by fragmentation. Type genus, *Chroococcus* Näg., *ibid.*, p. 45. 1848.

KEY TO GENERA

1. Cells spherical or discoid 2
   Cells ovoid, cylindrical, or pyriform 3
2. Cells dividing in three planes perpendicular to each other ..... Anacystis
   Cells dividing in two planes perpendicular to each other, plant laminate ..... Merismopedia
   Cells dividing in one plane, plant a uniseriate filament ..... Johannesbaptistia
3. Cells dividing in planes perpendicular to the long axes ..... Coccochloris
   Cells dividing in planes parallel with the long axes 4
4. Plant laminate ..... Microcrocis
   Plant spherical, the cells arranged radially at the periphery .. Gomphosphaeria

ANACYSTIS Menegh

Consp. Algol. Eugan., p. 324. 1837. —Type species, *A. marginata* Menegh., *loc. cit.* 1837.

KEY TO SPECIES

1. Cells without pseudovacuoles, plants not developing as water-blooms 3
   Cells containing pseudovacuoles, plants developing as water-blooms 2
2. Cells 3—6 (rarely 2—10)μ diam. ..... *A. cyanea* (Kütz.), comb. nov.[1]
   Cells 1—2μ diam. ..... *A. incerta* (Lemm.), comb. nov.[2]
3. Cells 1—1.5μ diam. ..... *A. nidulans* (Richt.), comb. nov.[3]
   Cells larger 4
4. Cells chiefly over 6μ diam. 5
   Cells 2—6μ diam. (larger where parasitized by fungi), the sheaths developing red, blue, or brown pigment in aerial situations ... *A. montana* (Lightf.), comb. nov.[4]
   Cells 2—4μ diam. (larger where parasitized by fungi), sheaths usually distinct and becoming colored in aerial situations ..... f. *montana*
   Cells 2—3μ diam., sheaths hyaline and diffluent, plants aquatic ..... f. *minor* (Wille), comb. nov.[5]
   Cells 5—6μ diam., sheaths hyaline and diffluent, plants aquatic ..... f. *gelatinosa* (Henn.), comb. nov.[6]
5. Cells chiefly 6—12μ diam., cells soon becoming spherical after division 6
   Cells 12—50μ diam., often remaining angular for long periods after division ..... *A. dimidiata* (Kütz.), comb. nov.[7]
6. Plants microscopic, in freshwater habitats 7
   Plants of indeterminate growth, often macroscopic, marine; protoplasm blue-green or red; homogeneous; sheaths diffluent ..... *A. aeruginosa* (Zanard.) Dr. & Daily
7. Sheaths narrow, protoplasm often sparsely large-granulate, plants chiefly subaerial ..... *A. thermalis* (Menegh.), comb. nov.[8]
   Sheaths broad and diffluent, plants planktonic ..... *A. limnetica* (Lemm.), comb. nov.[9]
   Cells widely spaced, not in compact cubical arrangement ..... f. *limnetica*
   Cells in compact cubical arrangement ..... f. *major* (Lagerh.), comb. nov.[10]

MERISMOPEDIA Meyen

Neues Syst. Pflanzen-Physiol. 3: 440. 1839. —Type species, *M. punctata* Meyen, *loc. cit.* 1839. Two species:

Cells 1—4μ diam., plants 2—64-celled ..... *M. tranquilla* (Ehrenb.) Trevis.
Cells 5—10μ diam., plants larger and often laminate ..... *M. thermalis* Kütz.

JOHANNESBAPTISTIA J. de Toni

Not. Nomencl. Algol. 1: 6. 1934. —Type species, *Cyanothrix primaria* Gardn., Mem. New York Bot. Gard. 7: 30. 1927.

A single species, the cells 4—20μ diam. ..... *J. pellucida* (Dickie) Taylor & Dr.

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[1] *Palmella cyanea* Kütz., Phyc. gener., p. 172. 1843.

[2] *Polycystis incerta* Lemm., Forschungsber. biol. Sta. Plön 7: 132. 1899.

[3] *Aphanothece nidulans* Richt. in Wittr. & Nordst., Alg. exs. 14: 694. 1884; Bot. Not. 1884: 128. 1884; Hedwigia 23: 66. 1884.

[4] *Ulva montana Lightf.*, Fl. Scot. 2: 973. 1777.

[5] *Aphanothece saxicola* β *aquatica* f. *minor* Wille, Öfvers. K. Sv. Vet.-Ak. Förh. 36(5): 22. 1879.

[6] *Aphanothece stagnina* f. *gelatinosa* Henn., Phyk. March. 1: 43. 1893; ex Forti, Syll. Myx., p. 77. 1907.

[7] *Trochiscia dimidiata* Kütz., Linnaea 8: 593. 1833.

[8] *Trochiscia thermalis* Menegh., Consp. Algol. Eugan., p. 334. 1837.

[9] *Chroococcus limneticus* Lemm., Bot. Centralbl. 76: 153. 1898.

[10] *Chroococcus helveticus* f. *major* Lagerh. ex Forti, Syll. Myx., p. 17. 1907.

COCCOCHLORIS SPRENG.

Fl. Halens. Mant. 1: 14. 1807. —Type species, *C. stagnina* Spreng., *loc. cit.* 1807. Four species:

Cells ovoid, 10—25μ diam., 1—3 times as long as broad..*C. aeruginosa* (Näg.), comb. nov.[11]
Cells ovoid, 4—8μ diam., 1.5—3 times as long as broad.....*C. stagnina* Spreng.
Cells cylindrical, 2—6μ diam., 3—8 times as long as broad..*C. elabens* (Bréb.) Dr. & Daily
Cells cylindrical, 1—3μ diam., 3—12 times as long as broad.....
.....*C. Peniocystis* (Kütz.) Dr. & Daily

GOMPHOSPHAERIA Kütz.

Alg. Aq. Dulc. Dec. 16: 151. 1836. —Type species, *G. aponina* Kütz., *loc. cit.* 1836. Three species:

Cells 3—5μ diam., containing pseudovacuoles; plants developing as water-blooms in fresh water .....*G. Wichurae* (Hilse), comb. nov.[12]
Cells 2—3μ diam., without pseudovacuoles.....*G. lacustris* Chod.
Cells 4—10μ diam., without pseudovacuoles.....*G. aponina* Kütz.

MICROCROCIS Richt. in Hauck & Richt.

Phyk. Univ. 11: 548. 1892. —Type species, *M. Dietelii* Richt., *loc. cit.* 1892.

One species, with cells 5—7μ diam.....*M. geminata* (Lagerh.) Geitl.

CHAMAESIPHONACEAE BORZI

N. Giorn. Bot. Ital. 14:298. 1882.—Plants uni- and multicellular, the cells separated from each other by sheath material; solitary cells spherical, ovoid, or cylindrical, basally attached to a substratum, dividing at first into daughter cells of unequal size and further developing (chiefly by cell divisions in planes parallel with the substratum) into cushions of radial structure, the basal cells often growing downward into the substratum; endosporangia formed from any cells which enlarge and divide internally, wholly or in part, into few or many endospores; reproduction by fragmentation or by endospores. —Type genus, *Chamaesiphon* A. Br. & Grun. in Rabenh., Fl. Eur. Algar. 2:148. 1865. One genus:

ENTOPHYSALIS KÜTZ.

Phyc. Gener., p. 177. 1843. —Type species, *E. granulosa* Kütz., *loc. cit.* 1843.

KEY TO SPECIES

1. Marine 2
   Freshwater 4
2. On rocks, wood, and shells; cells chiefly 4—6μ diam...*E. crustacea* (J. Ag.), comb. nov.[13]
   On larger algae or animals 3

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[11] *Synechococcus aeruginosus* Näg., Gatt. einz. Alg., p. 56. 1848.

[12] *Ceolosphaerium Wichurae* Hilse in Rabenh., Alg. Eur. 153—156: 1523. 1863; Hedwigia 1863: 151. 1863.

[13] *Myrionema crustaceum* J. Ag., Alg. Mar. Medit. & Adriat., p. 32. 1842.

3. Cells 1—2μ diam., plants yellowish in color.....*E. endophytica* (Howe) Dr. & Daily
Cells larger, plants blue-green or reddish.....*E. conferta* (Kütz.) Dr. & Daily
4. On rocks, wood, or shells
Basal cells ovoid.....*E. rivularis* (Kütz.) Dr.
Basal cells cylindrical.....*E. papillosa* (Kütz.) Dr. & Daily
On larger plants
Solitary cells spherical to cylindrical, not long-stipitate.....
.....*E. Brebissonii* (Menegh.) Dr. & Daily
Solitary cells spherical to linear-cylindrical and long-stipitate.....
.....*E. elongata* (Wille), stat. nov.[14]

CLASTIDIACEAE, fam. nov.[15]

Plants microscopic, solitary, epiphytic, cylindrical, basally attached to the substratum, at first unicellular, then dividing internally into a uniseriate chain of spherical cells which do not appear to be separated from each other by sheath material; sheath thin, closely investing the entire plant, enlarged at the base and adhering to the substratum; reproduction by the bursting of the sheath and the dissociation of the cells of the chain. —Type genus, *Clastidium* Kirchn., Jahresh. Ver. Vaterl. Naturk. Württemb. 36:195. 1880.

Two genera:

Plant terminating above in a hair-like extension of the sheath....
.....Clastidium
Plant smooth at the apex.....Stichosiphon

CLASTIDIUM KIRCHN. *loc. cit.* 1880.

Type species, *C. setigerum* Kirchn., *ibid.*, p. 196. 1880.

One species, with cells 2—4μ diam.....*C. setigerum* Kirchn.

STICHOSIPHON Geitl., in Rabenh.

Krypt.-Fl. 14: 411. 1931. —Type species, *S. regularis* Geitl., *ibid.*, p. 412. 1931.

One species, with cells 3—6μ diam.....*S. sansibaricus* (Hieron.), comb. nov.[16]

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[14] *Chamaesiphon gracilis* f. *elongata* Wille, Bih. t. K. Vet.-Akad. Handl. 8(18): 28. 1884.

[15] Plantae microscopicae, solitariae, epiphyticae, cylindricae, basim affixae, primum unicellulares demum interne in catenam cellularum sphaericarum uniseriatam dividentes; vagina tenue, ad apicem clausa, ad basem incrassata et substrato adhaerenti; reproductio dissolutione catenae cellularum et vaginae.

[16] *Chamaesiphon sansibaricus* Hieron. in Engler, Pflanzenw. Ostafr. C: 8. 1895.